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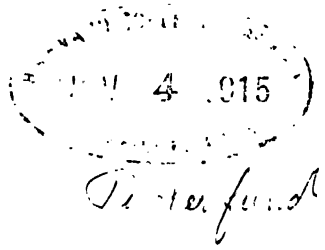
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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XXVII. THE MECHANISM OF REGURGITATION OF DUODENAL CONTENTS INTO THE STOMACH

CLARENCE J. HICKS, JR. AND JOHN W. VISHIER

From the Hull Physiological Laboratory of the University of Chicago

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Boldyreff's¹ statement that antiperistalsis in the duodenum is the mechanism of the regurgitation of intestinal contents into the stomach was the first instance in which antiperistalsis had been reported as a normal motor activity of any part of the gastro-intestinal tract, except, of course, the ascending and transverse colon. Accordingly, under the direction of Dr. Carlson, we attempted to verify this statement of Boldyreff's and to determine, if possible, additional facts bearing on this problem. The literature on this particular point appears to be confined to two papers by Boldyreff. In the first article² he says that when oil and acid are introduced into the stomach, duodenal regurgitation occurs by a "mechanism of its own." To support his later designation¹ of duodenal antiperistalsis as the mechanism of this regurgitation, he published no experimental data.

METHODS OF EXPERIMENTATION

I. Aseptic section of vagi and splanchnics in dogs, with comparison of regurgitation before operation and after recovery.

II. Surviving excised cats' stomachs with duodenum and jejunum in oxygenated Tyrodi's solution. Observation of movements when 0.5 per cent HCl was introduced into the stomach through oesophagus.

¹ Boldyreff: Quart. Journ. Exper. Physiol., 1914, viii, 8.

² Boldyreff: Ruskii Vrach, 1904, iii, 1305.

III. Fluoroscopic examination of cat's duodenum filled via jejunal fistula with BiONO_3 suspension, when 0.5 HCl was introduced into the stomach.

IV. Direct observation of movements of gastro-intestinal tract exposed under Tyrodi's solution at 37°C . and the effect upon these movements of the introduction of 0.5 HCl into the stomach through the oesophagus before and after the section of the vagi and splanchnics.

RESULTS

Method I of course required a series of experiments on a given dog before operation to determine the normal frequency of duodenal regurgitation when 0.5 per cent HCl had been introduced into the stomach. The procedure was as follows:

1. 150 cc. 0.9 per cent NaCl solution into stomach by oesophageal tube. Aspirated after 30 minutes—color noted.
2. 150 cc. 0.5 per cent HCl into stomach by tube. Aspirated after thirty minutes—color noted.

If the first removed sample was colorless, and the second showed any appreciable yellow tinge, this was considered a positive result; i.e., regurgitation had occurred. Whereas, if the recovered acid was colorless, it was considered a negative result.

It was decided that the color of the sample was a sufficiently delicate test for the presence of bile after it had been found that one drop of bile added to 100 cc. of water stained it a bright straw color.

It soon became apparent, however, that regurgitation under these conditions was the exception rather than the rule. Consequently nerve section was an unsatisfactory method of attacking the problem, because, with an irregular normal, it would be difficult to interpret the post-operative results. Accordingly this method was largely abandoned. We continued, however, to accumulate a large number of tests on normal dogs to determine in what percentage of cases regurgitation occurred under our stated conditions. The results were:

Using 15 dogs and testing them on an average of about five times each, a total of 76 trials:

150 cc. 0.9 per cent NaCl in stomach for thirty minutes caused no regurgitation in 100 per cent of 22 cases. Therefore, in later trials, the stomach was only washed with salt solution to determine possible presence of bile before the acid was introduced. 150 cc. of 0.5 per cent HCl in stomach for fifteen minutes = regurgitation positive in 6 out of 20 trials = 30 per cent positive. 150 cc. 0.5 per cent HCl in stomach

thirty minutes = regurgitation positive in 15 out of 36 trials = 43 per cent positive. The results of 20 additional trials were discarded because of emesis, or on account of the presence of bile in the stomach contents before the introduction of acid.

When regurgitation occurred, judging by the pale color of the returned acid, but very little duodenal contents could have entered the stomach. In spite of this fact, the acidity was usually reduced from 0.5 per cent to about 0.35 per cent HCl. This would seem to indicate that *duodenal regurgitation is not the factor of greatest importance in the reduction of the high acidity of the stomach contents*; for it is difficult to conceive of much pancreatic juice passing back through the duodenum without becoming mixed with bile.

Nine experiments on 4 dogs with sectioned splanchnics yielded 100 per cent positive regurgitation. In these cases the acid removed after thirty minutes contained much bile; and the acidity averaged only 0.237.

A man with a gastric fistula (Mr. V) being available, two series of tests were run to determine in what percentage of cases acid in the stomach caused regurgitation in man. These results are shown in the accompanying Tables I and II. In Table I it is seen that the appetite secretion of the gastric juice accumulated during twenty minutes' chewing food, and amounting to an average of 32.6 cc. with an average acidity of 0.411 per cent, caused regurgitation in 40 per cent of 10 cases.

Method II after five trials was also discarded because of the spasm of the pylorus induced, as described by Cannon,³ by section of the jejunum. This, of course, prevented the escape of gastric contents into the duodenum, and also made regurgitation impossible.

Method III (fluoroscopy) proved useless because the introduction of BiONO₃ suspension through a jejunal fistula caused pyloric spasm. Moreover, the bismuth, acting as a foreign substance itself caused duodenal contractions.

Method IV was followed on 11 cats and 1 dog with the following results.

1. 100 cc. 0.9 per cent NaCl introduced into the stomach via oesophagus induced no movements in the other duodenum and no regurgitation into the stomach.

2. In the 1 dog and in 9 out of the 11 cats 100 cc. 0.5 per cent HCl introduced into the stomach by tube via oesophagus induced rhyth-

³ Cannon, W. B.: Mechanical factors of digestion, p. 126.

TABLE I

Regurgitation of duodenal contents into stomach in man (Mr. V.) from the presence in the stomach of appetite gastric juice

DATE	200 cc. H ₂ O IN STOMACH ONE HOUR BEFORE CHEWING FOOD		APPETITE-SECRETION DURING 20 MINUTES CHEWING		REMARKS
	Amount color	HCl acidity	Amount color	HCl acidity	
1. July 19	18 cc. Very pale yellow	per cent 0.14 HCl	55 cc. Pale lemon color	per cent 0.407 HCl	Positive
2. 20	21 cc. Cloudy Opalescent	0.131	43 cc. Trace bile Clear opalescent	0.362	Faintly positive
3. 21	22 cc. Cloudy No bile	0.177	35 cc. Clearer Trace bile	0.462	Faintly positive
4. 22	17 cc. Very cloudy No bile	0.124	38 cc. Cloudy No bile	0.242	Negative
5. 23	24 cc. Colorless	0.096	28 cc. Colorless	0.411	Negative
6. 26	18 cc. Very cloudy No bile	0.255	40 cc. Fairly clear No bile	0.447	Negative
7. 27	26 cc. Cloudy (food?)	0.141	25 cc. Clear No bile	0.409	Negative
8. 29	18 cc. Opalescent	0.073	17.5 cc. Clear No bile	0.489	Negative
9. 30	20 cc. Clear	0.064	25 cc. Faint yellow	0.420	Positive
10. 31	21 cc.	0.130	30 cc. Clear—colorless	0.461	Negative
Average	20.3 cc.	0.134	32.6 cc.	0.411	40% Positive

TABLE II

100 cc. 0.4 per cent HCl into stomach of Mr. V. causes no regurgitation
(after 20 minutes)

DATE	200 cc. H ₂ O INTO STOMACH AT 10.00 A. M. EMPTIED AT 11.00 A. M.		100 cc. 0.4 % HCl INTO STOMACH AT 11.00 A. M. EMPTIED AT 11.20 A. M.		REMARKS
	Amount and color	HCl acidity per cent	Amount and color	HCl acidity per cent	
1. Aug. 9	12 cc. Clear Colorless	0.298	50 cc. Clear Colorless	0.395	Negative
2. 10	20 cc. Clear Colorless	0.306	24 cc. Clear Colorless	0.380	Negative
3. 12	11 cc. Clear Colorless	0.213	33 cc. Clear Colorless	0.362	Negative
4. 13	20 cc. Clear Colorless	0.266	39 cc. Clear Colorless	0.365	Negative
5. 17	21 cc. Cloudy Yellow	0.343	36 cc. Clear Colorless	0.351	Negative
6. 18	18 cc. Clear Colorless	0.252	41 cc. Clear Colorless	0.379	Negative
7. 19	25 cc. Clear	0.175	40 cc. Clear	0.369	Negative
8. 20	14 cc. Clear	0.237	34 cc. Clear	0.377	Negative
9. 23	16 cc. Clear	0.220	15 cc. Clear	0.319	Negative
10. 24	18 cc. Clear	0.241	26 cc. Clear	0.387	Negative
Average	17.5 cc.	0.2551	33.8 cc.	0.3629	100% Negative

mical pulsations and segmentation movements of the upper duodenum. In all of these animals regurgitation of duodenal contents into the stomach had taken place, as shown by the withdrawal of the stomach contents after thirty minutes.

3. In 2 of the cats the upper duodenum remained quiescent for at least thirty minutes after the introduction of 100 cc. 0.5 per cent HCl into the stomach, and the stomach contents of these two animals showed no intestinal regurgitation.

4. Actual antiperistalsis of the upper duodenum was never observed.

5. In the case of the 10 animals showing movements of the upper duodenum and intestinal regurgitation the average amount of the 100 cc. acid recovered after thirty minutes was 95.7 cc. with an acidity of 0.382 per cent. The 2 cats showing neither duodenal movements nor regurgitation yielded an average of 92 cc. with 0.44 per cent acidity.

6. In 5 cats with section of both vagi nerves in the neck 100 cc. 0.5 per cent HCl in the stomach caused similar movements of the upper duodenum and intestinal regurgitation in 4 cases, and no movements in the duodenum and no regurgitation in 1 case. In all these experiments the acid remained in the stomach thirty minutes.

7. In 4 cats with section of all the splanchnic nerves 100 cc. 0.5 per cent HCl in the stomach induced the above movements in the upper duodenum and regurgitation of intestinal contents in each case.

A brief description of the pulsating movements referred to in the above summary as invariably accompanying regurgitation and constantly absent when no regurgitation occurred, may be of interest. These movements occurred in the portion of the duodenum between the pyloric sphincter and a point just below the entrance of the common duct—a region corresponding with what Cole⁴ has named the "pilleus ventriculi." Here two types of movements were noted:

1. Multiple, periodic, deep, constricting rings occurring throughout this region, but mainly in the lower half of the first portion of the duodenum. These constrictions did not pass along as peristaltic waves; but rather resembled marked, slow, segmentation movements.

2. Peristaltic waves, usually deep, originating about 4 per minute in a pulsating ring just distal to the pyloric sphincter, and passing rather rapidly down to disappear in a tonic constriction just beyond the opening of the common duct.

Occasionally a third type of movement was noted in this region. The entire duodenum above the common bile duct was tightly con-

⁴ Cole: Jour. Amer. Med. Assoc., 1913, lxi, 762.

tracted as a whole, the walls appearing anemic—so strong was the constriction: This spasm lasted usually about one minute.

These movements began in from three to twenty-five minutes (average thirteen minutes) after the introduction of 0.5 per cent HCl into the stomach. They did not appear continuously throughout any experiment, but a period of gradually diminishing motor activity would be followed by a short period of rest.

Granting that the pyloric sphincter is relaxed occasionally (the presence of the strong acid on the gastric side should assure this) it is easy to see how any one of these three types of movements in the upper duodenum would be a mechanism capable of forcing back some duodenal contents into the stomach.

In general the amount of regurgitation appeared directly proportional to the degree and duration of the movements in the first portion of the duodenum. Antiperistalsis was never seen in the duodenum. This is in accord with Starling's⁵ and Cannon's statement that "an antiperistalsis is never observed in the small intestine." No movements of the upper duodenum were ever noted except as described above as invariably accompanying regurgitation following the introduction of acid into the stomach (or when 0.5 per cent HCl was introduced directly into the duodenum, as described in a succeeding paragraph.) This statement agrees with that of Holzknacht⁶ that peristalsis is rarely seen in the upper duodenum.

It may, of course, be objected that these observations were made upon animals under ether anaesthesia, and are therefore not truly normal. But a very light anaesthesia was always maintained. Moreover, the frequent observation of normal peristalsis in the stomach and small intestines, antiperistalsis in the ascending colon, and segmentation movements in the small intestine would seem to show that the motor activities of the gut were little impaired.

Having determined that the peculiar movements described above occurred following the introduction of 0.5 per cent HCl into the stomach even in the absence of extrinsic nervous connections, the following experiments were performed in an attempt to further localize the mechanism of regurgitation:

Cat under Tyrode's solution (as in Method IV).

⁵ Starling, E. H.: Recent advances in the physiology of digestion, Chicago, 1907, p. 142. Cannon, Loc. cit., p. 142.

⁶ Holzknacht and Jones: "Der Radiologische Diagnostik," etc., Wien. 1908, p. 17.

1. 100 cc. 0.5 per cent HCl into stomach per oesophagus.
2. Stomach emptied of acid contents and washed with 0.9 per cent NaCl soon after characteristic movements had started in the upper duodenum. (Regurgitation noted as faintly positive.)
3. After upper duodenum had become quiet and remained so ten minutes, 5 cc. 0.9 per cent NaCl was injected into the lumen of the upper duodenum with a very fine hypodermic needle. No movements of upper duodenum noted during following fifteen minutes.
4. 5 cc. 0.5 per cent HCl injected by needle into the lumen of upper duodenum. Characteristic pulsating movements noted after four minutes and continuing about ten minutes. Repetitions of this experiment on 3 other cats gave the same results.

This experiment shows that the discharge of the strong acid from the stomach into the upper duodenum initiates the movements in that region through local mechanisms.

CONCLUSIONS

I. 150 cc. 0.5 per cent HCl left in dogs' stomachs for fifteen and thirty minutes caused regurgitation in 38 per cent of 56 trials. In man, an average of 32.6 cc. gastric juice (acidity 0.411 per cent) accumulated during twenty minutes' chewing of food, caused regurgitation in 40 per cent of 10 trials. Whereas, 100 cc. of 0.4 per cent HCl retained in stomach for twenty minutes caused no regurgitation in 100 per cent of 10 other cases.

II. Duodenal regurgitation following the introduction of 0.5 per cent HCl into the cat's stomach is effected by means of characteristic movements (mostly "rhythmical pulsations" or "segmentation movements") in the first portion of the duodenum, initiated by the passage of strong HCl into the duodenum, and occurring in the absence of extrinsic nervous connections. Regurgitation never occurred in the absence of these movements.

III. Antiperistalsis of the duodenum is not concerned in this regurgitation.

⁷ Cannon: Loc. cit., p. 142.

THE ORIGIN OF THE PROTEOLYTIC FERMENTS OF THE BLOOD

THE QUESTION OF THE SPECIFIC CHARACTER OF CERTAIN FERMENTS

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From the Hull Physiological Laboratory of the University of Chicago

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Since the publication by Abderhalden (1) of his experiments on the "Protective ferments of the animal organism" and especially on the sero-diagnosis of pregnancy a very large literature has developed. The major part of this literature has concerned itself with the pregnancy reaction. The results obtained have been conflicting. Some workers have verified completely all data reported by Abderhalden while others have been unable to confirm the experiments.

The basis for the reaction is briefly this (2). Chorionic cells and substances not in harmony with the maternal blood get into the maternal blood stream. The presence of such cells and substances causes the development of an enzyme or ferment which is able to split placental protein.

Abderhalden and his students have steadfastly maintained the absolute specificity of the reaction. They contend that the blood of pregnant individuals contains a ferment which is specific for placental protein and that such an enzyme or enzymes with like power do not exist in normal blood serum or in the blood serum from other than pregnant source. Furthermore they believe the reaction to be a digestion of the substrate and not an autodigestion of the proteins of the serum by enzymes freed from an anti-enzyme inhibition. The recent work of Michaelis and Lagermarck (3), Flatow (4), Herzfeld (5) and of others abroad, and of Williams and Pearce (6), Falls (7), Jobling, Eggstein and Petersen (8) and Bronfenbrenner (9) in this country brings the position of Abderhalden into serious doubt.

From the work of these authors and others the existence of polyvalent enzymes in the blood serum is certain. These polyvalent enzymes are proteolytic in character. They may either be increased in amount

or in power of action under certain conditions. It is clear, therefore, that the value of the Abderhalden reaction will be apparent only when there is a quantitative differentiation between results obtained by the action of these enzymes and the so-called specific ferment of pregnancy.

The first worker to suggest this essential point was Kjaergaard (10) in May, 1914. In June, 1914 at the symposium on the subject before the American Medical Association (11) the same suggestion was made independently by Dr. Carlson.

It was with a view to reducing the Abderhalden reaction to a quantitative test and thereby to make its differential value greater that this investigation was carried out.

Early results showed that a differentiation on the strength of color produced with ninhydrin was unreliable. Ninhydrin is so easily affected by the physical and mechanical factors of the method as to make questionable the slight differences in depth of color. A greater or less color cannot always be assigned to a greater or less degree of digestion. The other quantitative method involved the time factor.

EXPERIMENTAL METHODS

In addition to the methods first suggested by Abderhalden, *i.e.*, the dialysis and optic methods (12), the following have since been suggested (a) Williams and Pearce coagulation method (*loc. cit.*), (b) the Van Slyke method (13), and (c) the very recent method of Kiutsi discussed and modified by Malone (14). Of these methods we have employed two: the simple method of Williams and Pearce and the dialysis method of Abderhalden.

The Williams and Pearce method was altered in but one respect. This involved the readjustment of the volume of the filtrate obtained after coagulation with acetic acid up to 15 cc. In the dialysis method thimbles No. 679a were boiled for fifteen seconds, inverted onto filter paper, allowed to cool and then handled with sterile forceps. One-half gram of the tissue desired was added and on to this from 1-1½ cc. of serum was allowed to flow from a sterile pipette. The contents was covered with a layer of toluol and placed in the special dialyzing flasks furnished by the Arthur H. Thomas Co. The outer liquid was covered with a thin layer of toluol and the whole incubated at 37-38° for a definite interval of time (16-20-44 hours). The controls varied. In every case an equal amount of serum was used as one control. At regular intervals all substrates were tested. Frequently substrate plus sterile

salt solution was used as control. In all the early tests with the Williams and Pearce method a control of placenta or other substrate and inactivated serum was used. In many instances all of these controls were run.

As to the preparation of the substrate some change was made from Abderhalden's directions purely in the way of safeguarding the reaction. Abderhalden advises boiling the substrate until a test with 5 cc. of the clear filtrate fails to give a reaction with 1 cc. of a 1 per cent solution of ninhydrin. We boil the material three times after the test is no longer given for about five minutes each time but do not, however, continue to boil beyond seven times. On removal from the containing jar the tissue is washed with distilled water, pressed between filter paper and then weighed. The requisite quantity of tissue is put into a beaker and boiled twice with about 50-75 cc. of water. Between boilings the tissue is rinsed with distilled water and after the second boiling is placed upon filter paper. Sterile sheets cover all working surfaces. Other details are as given by Abderhalden.

EXPERIMENTS

These consisted of the following series.

- A. Action of blood serum from pregnant individuals on human placenta.
- B. Action of blood serum from pregnant individuals on various tissues.
- C. Action of blood serum from normal individuals on human placenta.
- D. Action of blood serum from various pathological conditions on human placenta.

A. THE ACTION OF BLOOD SERUM FROM PREGNANT INDIVIDUALS ON HUMAN PLACENTA

The results of the tests on serum from 14 pregnant individuals using the Williams and Pearce technic are summarized in Table I. Tests were made with serum from 23 other pregnant individuals using the dialysis method with the results given in Table II. An additional series of tests were made on a pregnant goat with the results shown in Table III.

TABLE I

Action of blood serum from pregnant women and pregnant dogs upon human placenta using the Williams and Pearce technic*

SOURCE OF SERUM	NO. OF TESTS	POSITIVE	NEGATIVE	TIME
Women in ninth month.....	10	10	0	hours 20
Dogs 1-5 days before term..	4	4	0	20

*The preparation of a satisfactory substrate from the placenta of dogs is very difficult. Following a suggestion of Abderhalden (15) human placenta has been used throughout.

TABLE II

Action of blood serum from pregnant women and pregnant dogs upon human placenta using the dialysis method

SOURCE OF SERUM	NO. OF TESTS	POSITIVE	NEGATIVE	TIME
Women in ninth month.....	5 excl. of pre- liminary tests	5	0	hours 20
Healthy pregnant dogs.....	18	16	2	20-24

In one case a series was run on dog on arrival at laboratory. Serum very fatty. Absolutely negative results.

TABLE III

Action of the blood serum of pregnant goat upon human placenta, extending over 7½ weeks. Dialysis method

DATE	AMOUNT OF SERUM	NO. OF TESTS	POSITIVE	NEGATIVE	TIME
February 22....	cc. 1	4	4	0	hours 20
March 11.....	1	1	0	1	20
March 15.....	$\begin{Bmatrix} 1 \\ 1 \end{Bmatrix}$	$\begin{Bmatrix} 1 \\ 1 \end{Bmatrix}$	$\begin{Bmatrix} 0 \\ 1 \end{Bmatrix}$	$\begin{Bmatrix} 1 \\ 0 \end{Bmatrix}$	$\begin{Bmatrix} 20 \\ 40 \end{Bmatrix}$
April 14.....	$\begin{Bmatrix} 1 \\ 1.5-2 \end{Bmatrix}$	$\begin{Bmatrix} 1 \\ 2 \end{Bmatrix}$	$\begin{Bmatrix} 0 \\ 2 \end{Bmatrix}$	$\begin{Bmatrix} 1 \\ 0 \end{Bmatrix}$	$\begin{Bmatrix} 20 \\ 20 \end{Bmatrix}$

*Two kids were born on April 17

B. THE ACTION OF BLOOD SERUM FROM PREGNANT INDIVIDUALS ON VARIOUS TISSUES

The tissues used were prepared by transfusing the dogs with normal salt solution until the organs were practically blood free and then grinding and boiling as given in Abderhalden's directions. The results are summarized in Table IV.

TABLE IV

Action of blood serum from pregnant women, pregnant dogs and a pregnant goat on dog pancreas, liver and kidney

TISSUE	NUMBER OF TESTS	AMOUNT OF SERUM	POSITIVE	NEGATIVE	TIME
		cc.			
Pancreas.....	{ 3 human 7 dog	1.5	{ 3 6	1	20-22
Liver.....	{ 1 goat 5 dog	1.5	5	1 dog	20-22
Kidney.....	4 dog	1.5	1	3	20-22
Summary*.....	20		15	5	

*Excludes dog which gave negative with placenta. All reactions with this same serum on pancreas, liver and kidney were negative.

In the series some of the tests were run parallel with those upon placenta. Opportunity was thus afforded to compare the strength of reaction. In the majority of instances placenta gave the deeper color. While this might indicate a specificity of a definite sort, still, as mentioned before, too much importance cannot be put upon mere depth of color.

It seemed especially important at this point to determine (a) whether there exist in the blood serum of *normal* individuals enzymes which can produce dialyzable products with placental protein and (b) if such enzymes do exist can we differentiate between the polyvalent enzymes and the specific enzymes of pregnancy by any quantitative method.

C. THE ACTION OF BLOOD SERUM FROM NORMAL INDIVIDUALS ON HUMAN PLACENTA

It was later found that the method we employed had been used previously by Kjaregaard (loc. cit). It involved the extension of the

time of incubation from twenty to forty-four hours. The limit of forty-four hours was placed after carrying out a series of tests for twenty, twenty-four, thirty, thirty-six, forty, forty-four-hour periods. The results are given in Table V. Similar tests were carried out using dog kidney, liver and pancreas as substrate with practically identical results.

TABLE V

Action of blood serum of normal individuals on human placenta for 20 to 44 hour periods

SOURCE OF SERUM	NUMBER OF TESTS	TIME	POSITIVE	NEGATIVE
		<i>hours</i>		
Male dogs.....	14	20-22	0	14
Male dogs.....	16	40-44	14	2
Human male....	4	20	0	4
Human male....	4	40-44	4	0

D. THE ACTION OF BLOOD SERUM FROM VARIOUS PATHOLOGICAL CONDITIONS ON HUMAN PLACENTA

As the final step in the investigation a series of experiments was run on dogs in various pathological conditions in order to determine whether these involve an increase in the non-specific serum enzymes. The results are given in Table VI. In conditions I and III the dogs tested negative before operation. The increase in ferment activity

TABLE VI

Action of blood serum from various pathological conditions on human placenta

CONDITION	NUMBER OF TESTS	RESULT
I. Cachexia, pancreatic diabetes.....	4	All of the tests were positive at one stage or another
II. Acute distemper.....	2	
III. Jaundice.....	7	
IV. Pneumonia.....	1	
V. Ulcer of stomach and distemper.....	2	
VI. Generalized infection.....	1	

was clearly evident in these animals. When the loss of weight began to be noticeable there was an increase in the enzyme strength as shown by the decreased time of incubation necessary to give a positive test. When this loss in weight became very rapid the test was always given within the specified time of the Abderhalden reaction.

DISCUSSION

It would seem that the data presented in Tables I, II, and III can be explained on either of these three hypotheses (a) a specific enzyme capable of acting upon placental protein matter; (b) an increased concentration of non-specific enzymes which digest placental protein; plus the action of specific enzymes; or (c) enzymes which act upon the proteins of the serum and yield dialyzable products. *Regardless of the mode of action, there is a definite increase in the proteolytic ferment strength of the blood serum during pregnancy.* This condition is not limited to human pregnant but is also found in pregnant dogs, goats, and in other animals (16). As mentioned before, Abderhalden believes that this increase in ferment activity is of a specific character and is developed in response to a specific protein antigen. He assumes that because of this specific character the enzyme acts only upon placental proteins or proteins of very similar structure. There is nothing in our results to substantiate this view. That is to say, the increased proteolytic power of pregnancy serum may be due to other factors.

In Table IV we have presented results which show that the Abderhalden reaction is more intricate than at first supposed. The results observed here were so constant as not to be due to errors in technic. It will be noted that the kidney was refractory to the action of blood serum from pregnant individuals. This refractory character we cannot explain at present. It is not attributable to the use of one preparation, as, new preparations were made several times and the same refractory character seen. It is clear, therefore, that in pregnant as well as in non-pregnant mammals there exists one or more non-specific enzymes or a number of specific enzymes capable of acting upon protein tissue substrates and producing dialyzable products. Similar results have been obtained by previous investigators. In the Abderhalden test we are dealing either with a production of new, non-specific enzymes or an increase in the power of enzymes already present. It is very likely that a highly specific enzyme does exist in the blood of pregnant, but if so, the dialysis method as developed now and the

Williams and Pearce method do not serve to separate its action from the action of the nonspecific enzymes which are certainly present. In other words the coagulation method does not afford us a means of procuring a proper substrate for the supposed specific enzyme to confine its action to. Furthermore, on the basis of Table V, it is most reasonable to suppose that what we are dealing with is an augmentation of the digesting powers of enzymes already present in the blood preceding the period of gestation; i.e., that we have a quantitative increase. This increase in the action of the non-specific enzymes *may* be further augmented by the presence of a specific enzyme. In Table V it is clearly shown that non-specific enzymes do exist in normal blood serum. This is not a new fact, but a fact in some instances denied, and in no instance sufficiently emphasized by Abderhalden and his pupils.

In 1904 Hedin (17) showed that the blood serum of a normal ox has a weakly proteolytic action on casein and gelatin in alkaline solution. The ferment or enzyme was held apparently in the globulin fraction. At the same time Delezenne and Pozerski (18) brought forward evidence to show that blood serum incubated under chloroform gained in proteolytic power. More recently Kjaergaard has shown this proteolytic power of normal blood serum. The conception has been clearly stated most recently by Vaughn (19).

In a communication to the Chicago Pathological Society (article not yet published), Falls has given further evidence that during digestion the ferment power of the serum of the portal blood is greatly increased when compared with that of the peripheral blood (femoral). If the portal blood does show this enzyme increase during digestion it is highly probable that some of these are present in the normal serum at all times.

Our method for the detection of these enzymes was simply extending the time of the incubation. Some of the tests were so arranged that the final step was performed at the same time for twenty and forty-four hour samples. The marked difference in the reactions left no doubt as to the greater degree of dialysis and consequently the greater amount of digestion in the specimen incubated more than twenty hours. It will be noted that in no case was a positive reaction obtained in twenty hours upon normal serum whereas in only two cases was the test doubtful at the end of forty-four hours. Of the other tissues used kidney was again refractory. The tests are not explicable on the basis of fetal remnants since males were used. In the application of the method

to normal blood it was found that in practically every case there was a reaction in forty-four hours. In a few cases the reaction was apparent in thirty-six hours but rarely before. From this fact we feel safe in assuming that the differentiation of *normal* and pregnant blood can be made on the basis of the present test using the specified time of sixteen to twenty hours as the period of incubation and if the test is positive the individual is pregnant or is subject to some pathological condition.

A view recently presented by Jobling, Eggstein and Petersen (*loc. cit.*) assigns the mode of action of the ferments in pregnancy to an adsorption of antitrypsin which permits the auto-digestion of the proteins of the serum. Bronfenbrenner believes that the action is a sensitization of placenta by the serum. If the reaction is purely one of adsorption we should have expected positive tests with kidney as substrate. Some believe the reaction to be a typical antibody production. If so we should expect it to be a specific reaction. In our hands this is not the case.

In this connection the experiments on the goat are interesting. Our results show a considerable decrease in ferment activity even previous to the birth of the kids. Whereas on February 22, 1 cc. of serum was sufficient to produce a positive reaction in four cases, on March 11 the same amount did not give a positive reaction. Neither did this amount give a positive test on March 15 or April 14. The interpretation of this is not clear. It may be that some animals reach a condition even previous to parturition in which they can no longer produce the bodies giving to the serum of pregnancy its proteolytic power. In this condition they would resemble those "animals which under the continuous influence of antigen eventually lose the power to produce antibodies" (Hektoen, Harvey lectures, January 15, 1910).

In October of 1914, Falls (*loc. cit.*) reported a series of experiments upon the reaction of placenta with serum from certain pathological conditions. He concluded that "in many conditions of altered metabolism . . . there is an abnormal amount of ferment in the blood." Others have reported similar results. Recently King (20) has reported an increase in the diastases of the blood during wasting conditions. Our results confirm the work of Falls (Table VI). Since in normal serum we have non-specific ferments present invariably it is reasonable to suppose that in the conditions here cited we are dealing with an increase of these same enzymes, especially if such conditions are induced without the presence of an infectious organism. In the

condition of acute pancreatic diabetes and jaundice we are very definitely dealing with a quantitative increase in enzyme strength.

In the early stages of the condition noted above the differentiation on a time basis between pregnancy was easy since no reaction was given in twenty hours. When the condition had advanced this method was of no avail. We believe, therefore, that this method of differentiation between pregnancy and certain pathological conditions is of little value especially if the pathological processes are far advanced and of a wasting character. In other words, the non-specific enzymes are so increased as to mask the results of a specific enzyme if it be present (as in pregnancy).

We believe that at the present time the Abderhalden method does not provide a reliable test for the differential diagnosis between the strictly physiological state of pregnancy and certain pathological conditions.

CONCLUSIONS

I. There is an increased proteolytic ferment action of the blood serum during pregnancy.

II. This increased activity is probably in the main due to an increase in the polyvalent ferments.

III. Normal blood serum has a weak non-specific proteolytic action.

IV. The dialysis method of Abderhalden or the method of Williams and Pearce does not suffice to demonstrate a ferment developed during pregnancy acting only upon placental protein.

V. Many advanced pathological conditions give a positive reaction within the time specified for the Abderhalden test of pregnancy.

VI. At present the Abderhalden test is a quantitative not a qualitative test.

Thanks are due Dr. Carlson for many helpful suggestions during the course of this work.

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THE OXYGEN AND CARBON DIOXIDE CONTENT OF THE BLOOD DURING HIBERNATION IN THE WOODCHUCK (*MARMOTA MONAX*)

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INTRODUCTION

Of the several theories attempting an explanation of the cause of hibernation in mammals, a most interesting one, especially in the light of renewed work on the rôle of CO_2 in the dormancy of plants (1), is the theory that so-called winter-sleep is due to the accumulation of CO_2 in the blood and tissues of the animal. This excess of CO_2 is supposed to cause a form of narcosis as seen in the torpid condition of the hibernating animal. When the CO_2 reaches a certain concentration, the respiratory center is excited, respiration accelerated and the muscles become hyper-irritable. These culminating results are responsible for the awakening from dormancy.

A suggestion of this theory is found in the work of Bert (2), who in 1873 concluded that the dormancy may be due to the accumulation of CO_2 rather than a diminished supply of oxygen, as he had earlier believed. It was, however, apparently worked out independently as the *carbonic auto-narcosis* theory by Dubois (3) (1895). It appears that the attention of Dubois was called to the researches of Bert by Gley (4) some time after Dubois had published his carbonic auto-narcosis theory of natural sleep.

Dubois claims (5) that he can induce typical hibernating sleep by causing the active marmot to breathe a mixture of air (43 per cent), CO_2 (45 per cent) and oxygen (12 per cent). Further, he shows that torpid marmots remain dormant if supplied with this mixture; but by increasing the amount of CO_2 such animals may double their rate of respiration in ten minutes and may be awakened apparently as they naturally awake from lethargy. The author maintains (6) that the CO_2 acts principally on a nervous center for sleep and awakening situ-

ated in the mid-brain, since marmots deprived of cerebral hemispheres can sleep and awake, but with the bulb (medulla), only, in tact they are unable to wake up.

In support of the above conception Dubois showed (7) (1894), for the first time, that CO_2 actually accumulates in the blood during hibernation of the marmot and decreases again when the animal wakes up and becomes active. He found, on the other hand, but little difference in the amount of oxygen in the blood during the two states.

Dubois worked with the common European marmot (*Marmot vulgaris*). The figures given by him show that there is 15.4 cc. to 18.06 cc. of oxygen per 100 cc. of arterial blood after two to ten days of torpidity. In the normal active animal he found 15.3 cc. of oxygen in the arterial blood. In the venous blood there is only 6.05 cc. of oxygen after ten days of lethargy, as compared with 8.75 cc. during activity. The quantity of CO_2 is always greater in the blood of the marmot than in the blood of the rabbit, which was used as a control, the difference being especially great when the marmot is hibernating. In the marmot during winter-sleep there is from 63.23 cc. to 76.16 cc. of CO_2 per 100 cc. of arterial blood after two to ten days of torpor, as compared with 41.33 cc. during the active state. In the venous blood after ten days of torpor there is 74.05 cc. of CO_2 , as compared with 52.33 cc. during activity. The nitrogen, which amounted to about 2 cc. in each case, does not concern us here.

In regard to the decrease in CO_2 in the blood upon awakening from lethargy, Dubois (8) gives the average results obtained by the analysis of four samples of arterial blood taken from marmots (the number is not stated) which had awakened naturally during the hibernating period, as follows (in cubic centimeters per 100 cc. of blood): $\text{O} = 14.35$, $\text{CO}_2 = 52.3$, $\text{N} = 2.8$, total gas = 69.45. The corresponding figures for three samples of venous blood are: $\text{O} = 7.8$, $\text{CO}_2 = 53.5$, $\text{N} = 2.6$, total gas = 63.9. He also gives the following figures as the result of analysis of the blood of two marmots artificially awakened during winter and taken to a warm place where they were fed for eight days: $\text{O} = 14.55$, $\text{CO}_2 = 57.37$, $\text{N} = 2.7$, total gas = 74.62 in arterial blood; and in the venous blood, $\text{O} = 6.63$, $\text{CO}_2 = 56.1$, $\text{N} = 3$, total gas = 65.73. Finally, the figures resulting from the analysis of blood taken from marmots (the number is not stated) in summer after feeding for five weeks, are: $\text{O} = 15.66$, $\text{CO}_2 = 48.4$, $\text{N} = 1.6$, total gas = 65.66 in arterial blood; and in venous blood, $\text{O} = 11.13$, $\text{CO}_2 = 55.3$, $\text{N} = 2.26$, total gas = 68.69.

Dubois further shows that the blood of the torpid marmot has a greater capacity for the absorption of CO_2 and oxygen than has the blood of the active animal, due, he thinks, to the greater specific gravity of the blood during lethargy.

The gases in the above analyses were obtained by means of a mercury pump, and were analysed in a eudiometer tube. Nothing is said about the method of obtaining the blood.

From a rather extensive examination of the literature, these facts obtained by Dubois appear to be all that exist on the subject of blood gas analysis during hibernation. Some general observations on the appearance of the blood during this condition are on record. Saissy (9) (1808) observed that the blood during torpor is reddish brown in the arteries just as it is in the veins. Prunelle (10) (1811) found the arterial blood in the bat brighter red than the venous, but darker than ordinary arterial blood. Marshall Hall (11) (1832) said that the blood becomes venous. On the other hand, Valentin (12) (1865) states that in profoundly dormant marmots the venous blood is nearly as red as the arterial and is a cherry red, reminding one of the blood of reptiles and embryos. Quincke (13) (1882) agrees with this observation. Claude Bernard (14) (1859, 1878) and Marès (15) (1892) similarly state that all the blood is arterial during hibernation. A more recent observation is recorded by Allen Cleghorn (16) (1910), who says that he can confirm the previous observations on the arterial character of the blood, for he finds that it has an arterial or bright red hue in the veins. These conflicting statements give no real information as to the actual gas content of the blood during the two states of the animal. In the woodchucks used in the analyses to be recorded here, there was found to be a very pronounced difference in the color of the arterial and the venous blood during the torpid state. The arterial blood is bright red and the venous blood is dark brown, ordinarily, both during lethargy and during activity. But in animals that are being excited and are waking up, the venous blood may become nearly as bright as the arterial. This may be due to the greatly increased circulation and respiration, and may account for the above observations on the arterial character of the blood during winter-sleep.

PRESENT INVESTIGATION

Since actual gas analysis of the blood of hibernating animals is limited to the few determinations made by Dubois, and since Mosso (17) (1899) has an opposing acapnia theory according to which hibernation is due

to the lack of CO_2 in the system, the present investigation was undertaken to see what conditions in this particular occur in the woodchuck. It is well known that the woodchuck (American marmot) is one of the best examples of a hibernating mammal in this country. All species go into winter-sleep from four to six months each year (18). In this vicinity (Ithaca, New York) they seem to retire, as a rule, by the beginning of November, and they are seen again running about in March. They do not store any food in their burrows. In captivity, at least, they may wake up a few times during the winter. They are occasionally found sufficiently awake to retreat slowly to some other part of the burrow when exposed to the cold air. Most of the time, however, they are very dormant.

The animals used in this study were captured uninjured in the last week of August and first week of September, 1914, in this neighborhood. Six of them were kept in an open dog court at the laboratory in a box, 43 x 30 inches and 30 inches deep, with an extra bottom, 6 inches above the main bottom, so as to raise the animals above the ground. This box is lined with expanded iron and filled with straw. It communicates with a feed box of about the same size, lined with galvanized sheet iron, and covered by a lid of expanded iron. This feed box was removed on November 21, 1914, when the woodchucks commenced to hibernate, and the animals were shut up in the straw-filled box, which in turn was inclosed in a wooden structure, 6 feet 4 inches x 4 feet 6 inches and 4 feet high and covered by a roof of corrugated iron. The space between this enclosure and the box containing the woodchucks, was packed with dry straw. In this manner the animals hibernated very well till the end of February when the last one of this lot was killed. The rectal temperature of these animals between December 5 and February 27 varied from 6°C . to 14°C .

The rest of the woodchucks were kept in three of the eight artificial burrows mentioned by Simpson (19) three years ago. Since few details were given at that time, the opportunity for a more detailed description is taken here.

Each burrow consists of a sheet iron cylinder, 19 inches in diameter, 5 feet deep, closed at the bottom and covered by a removable tin lid on top. It projects 8 inches above the ground. The wall is perforated with $\frac{1}{2}$ inch holes, 6 inches apart. Near the bottom is a hole large enough to admit the end of a 6 inch glazed tile pipe, which communicates with a central pit or basket of expanded iron, 42 inches in diameter. The bottom of this pit is a little lower than the bottom of the burrow,

just described, and the top is open. Covering this surface opening is a gabled roof of corrugated iron, 60 inches x 51 inches. An opening near the surface of the ground permits the animals to enter the pit and the burrows, or to go out to the surface of the ground at will. The eight burrows encircle this central pit, 20 inches distant. The whole is surrounded by a galvanized sheet iron enclosure, $14\frac{1}{2}$ feet x $14\frac{1}{2}$ feet and 6 feet 8 inches high, floored with expanded iron 10 inches below the surface of the ground. This enclosure is covered overhead by heavy wire netting to keep out dogs, etc., since it is located about half a mile from the laboratory on the slope of a small hill. The central pit and the burrows are filled with dry straw.

The torpid animal is always found near the bottom of a burrow in a nest of dry straw. It will be seen that the upper portion of the dormant animal is thus about 48 inches below the surface of the ground. This, apparently, is about the depth at which these animals naturally hibernate, according to the description of the natural burrows given by Fisher (20).

All the animals were given fresh water and food, such as green clover, corn, apples and carrots, every other day till November 19, 1914. From that date no water or food was obtainable by these animals till after hibernation. Feeding was not recommenced till April 10, 1915, on account of the late spring. Woodchucks have hibernated well in these artificial burrows every winter for several years.

METHOD

At various periods preceding, during, and after hibernation, animals were taken and disturbed as little as possible while the blood samples were rapidly drawn. If the animal was active it was rapidly etherized and tied to an animal board. In one case chloretone was used as the anaesthetic in order to check the other results. If the animal was torpid no anaesthetic was used (with one exception to be mentioned later) since it is possible, by keeping the dormant animal cool either by operating in winter near an open window or by packing the animal with snow, to operate for an hour or longer without disturbing it much. The femoral artery was then isolated ready for the insertion of a cannula. The external jugular was next isolated and a small T tube, with the short limbs minimal in length, was inserted near the lower end or base of the vein so as to drain from both directions. In this way it was possible to get blood enough from the vein, notwithstanding the low blood pressure encountered, especially during hibernation. After allowing the blood to wash out the rubber connections attached to the cannula,

from 3 cc. to 4 cc. of venous blood was collected and as soon as possible (within five minutes from the time bleeding was commenced) placed under the ammonia solution in the bottles of the apparatus. Then an ordinary cannula was inserted into the femoral artery and a similar quantity of arterial blood was collected in the same way and immediately placed in the apparatus. The whole operation of getting the blood samples was accomplished as a rule in forty minutes or less.

The analysis was carried out by the chemical method of Haldane (21) with the apparatus devised by Brodie (22), which was calibrated by the method of Hoffmann (23). Five manometers were used for each sample of blood in order to reduce mechanical errors. Due to the small quantity of blood in the animal and the desirability of getting samples both of arterial and of venous blood as rapidly and as near together as practicable, with the least possible disturbance of the animal, it was deemed better to get one large sample of each kind of blood rather than several smaller ones. The average of the five determinations made on each sample was taken as the amount of gas in the blood.

RESULTS

The results and other data relating to the animals, are recorded in the table below.

ANIMAL (SERIES II)	DATE OF ANALYSIS	GAS IN CC. PER 100 CC. OF BLOOD REDUCED TO 0°C. AND 760 MM. PRESSURE			CONDITION OF ANIMAL	
		A = Arterial V = Venous D = Difference	O ₂	CO ₂	Rectal Temperature—C.	Anaesthesia, etc.
3	Sept. 28, 1914	A	17.82		35.8	Active, before hibernation, ether anaesthesia
		V	11.85			
		D	5.97			
4	Oct. 17, 1914	A	24.01	60.69	36.4	Active, before hibernation, ether anaesthesia
		V	19.64	64.28		
		D	4.37	3.59		
5	Nov. 7, 1914	A	22.60	75.06	33.9	Active, before hibernation, ether anaesthesia
		V	13.35	76.31		
		D	9.25	1.25		

ANIMAL (SERIES II)	DATE OF ANALYSIS	GAS IN CC. PER 100 CC. OF BLOOD REDUCED TO 0°C. AND 760 MM. PRESSURE			CONDITION OF ANIMAL	
		A = Arterial V = Venous D = Difference	O ₂	CO ₂	Rectal Temperature—C.	Anaesthesia, etc.
6	Dec. 5, 1914	A	28.79	87.69	14	Asleep, just commenced to hibernate, ether which woke animal up and greatly excited respiration
		V	18.52	93.20		
		D	10.27	5.51		
7	Dec. 19, 1914	A	25.76	68.80	6	Torpid, no anaesthesia
		V	6.16	96.00		
		D	19.60	27.20		
8	Jan. 16, 1915	A	23.08	43.01	6 but rose to 20	Torpid, no anaesthesia. Woke up before blood was obtained, respiration increased from 11 to 34 per min.
		V	12.93	71.24		
		D	10.15	28.23		
10	Feb. 6, 1915	A	22.20	83.06	6	Torpid, no anaesthesia
		V	6.65	98.08		
		D	15.55	15.02		
11	Feb. 27, 1915	A	20.23	86.39	9	Torpid, no anaesthesia
		V	13.53	100.64		
		D	6.70	14.25		
12	Mar. 20, 1915	A	16.09	76.40	25	Awake but rather sluggish, torpid 6 days previous, ether anaesthesia
		V	11.52	79.18		
		D	4.55	2.78		
13	April 10, 1915	A	12.39	74.61	36	Been awake for about 3 weeks, nothing to eat, chloretone per rectum
		V	7.09	80.56		
		D	5.30	5.95		
14	May 18, 1915	A	14.83	59.30	37.2	Been awake for 2 months, fed for 5 weeks, ether anaesthesia
		V	8.86	60.57		
		D	5.97	1.27		

It will be noted that there is an increase of about one-third in CO_2 during hibernation. This agrees with the results obtained by Dubois on the European marmot. The increase is especially marked in the case of the venous blood, where the CO_2 amounts to over 100 vol. per cent in the latter part of torpidity. The difference between the amount of CO_2 in the venous blood and in the arterial blood reaches its highest at about the middle of the period of torpidity. This great difference may be due to greater respiratory exchange as a result of slow circulation. Upon waking up there is a fall in the CO_2 in the blood as was also noted by Dubois. This fall is especially marked in the venous blood. At all stages included in this period of observation, there is a larger amount of CO_2 than is normally the case in most mammals, a fact also observed by Dubois in case of the European marmot.

In case of animal No. 8 we failed to get the venous blood to flow from the jugular on the left side and hence had to go to the right side, where the operation was also attended with some difficulty. In the meantime the animal woke up before any blood was obtained. During the one hour and twenty minutes consumed in getting blood, respiration increased from 11 to 34 per minute and the rectal temperature rose from 6°C. to 20°C. The increased respiration of the animal necessarily vitiates the results. It appears that the amount of CO_2 in the blood was greatly reduced, especially in the arterial blood. This sudden fall in CO_2 in the blood would contribute to the increased R. Q. observed by many (24) in hibernating animals as they wake up. The oxygen in the venous blood was evidently increased to about twice the amount usually found during torpor in the woodchuck.

Another irregularity in the series is found in connection with animal No. 6. This woodchuck had just become dormant as is evident from the date and the rectal temperature. Ether was given to prevent it from waking up and struggling. The ether greatly excited the animal, increasing the respiratory movements and waking it up while the blood samples were being taken. A high oxygen content but not a low CO_2 content curiously resulted.

The amount of oxygen in the blood varies much more than is indicated by the figures given by Dubois. The results here show a much higher percentage of oxygen, especially in the arterial blood, in the woodchuck during and just preceding winter-sleep (amounting to 26 vol. per cent in animal No. 7) than was found in the European marmot. In view of the very recent work by Christiansen, Douglas and Haldane (25) (1914) on the absorption and disassociation of CO_2 by blood, show-

ing that oxygen tends to drive out CO_2 and that the action depends upon the saturation of the hemoglobin, the large amount of CO_2 in the presence of a high percentage of oxygen indicates other changes in the blood of the woodchuck during hibernation.

Another evident fact is the great difference between the amount of oxygen in the arterial blood and in the venous blood during torpidity. This difference amounts to 19 vol. per cent in animal No. 7. This may also be due to slower circulation and the resulting greater respiratory exchange.

Aside from the bearing these facts may have on the carbonic auto-narcosis theory of Dubois, they suggest that the hemoglobin or other components of the blood undergo some marked changes which may either have something to do with the onset of hibernation or are the results of the lethargy. The apparent increase in the oxygen and CO_2 absorbing capacity of the blood during winter-sleep, as was indicated by Dubois (26) and which he attributed to an increase in the specific gravity of the blood (27), needs further investigation because facts which have not yet been published indicate that there is no marked increase in the specific gravity of the blood in the woodchuck during hibernation.

A criticism may be offered from the fact that ether was generally used as an anaesthetic on the active animals, while no anaesthetic was used on the torpid ones, except as was mentioned above in connection with animal No. 6. The ideal condition for this experiment would be to get the blood from the normal animal at all times. The active woodchuck, however, is vicious and must be immobilized. Chloretone, which in general is fairly satisfactory, seems to take effect too slowly when given per rectum even in large doses. It is practically impossible to get the drug into the stomach in this case since the animal can be handled only by suspending it by its tail. The least disturbance of the respiration of the active woodchuck was found to result from placing the animal in a box where the air was already saturated with ether. In no case—except in No. 6, where the animal was really dormant—was there a noticeable increase in respiration due to ether, although ether is generally considered to be a respiratory excitant and tends to increase the amount of oxygen and decrease the amount of CO_2 in the blood (28). The rather low per cent of oxygen in the blood of the active animal would seem to indicate that there was little if any over ventilation. The result in the case of animal No. 13 where chloretone was used (chloretone being considered a depressant), also indicate that but little error has crept in because of the use of ether.

SUMMARY

1. The amount of CO_2 in the blood of the woodchuck is at all times great as compared with that of most of the other mammals whose blood has been analysed.

2. The amount of CO_2 increases progressively during hibernation and decreases again when the animal wakes up.

3. There is also a larger per cent of oxygen in the arterial blood just preceding and during torpidity than at other times.

4. The difference between the amount of CO_2 in arterial and that in the venous blood is much greater during hibernation. There is also generally a greater difference between the amount of oxygen in the arterial and that in the venous blood during the dormant state.

I wish to thank Dr. Sutherland Simpson for his kind help and many suggestions during the progress of this work. I am also indebted to Miss A. E. Kühner for assistance in many of the operations.

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BLOOD COUNTS IN THE FROG, THE TURTLE AND TWELVE DIFFERENT SPECIES OF MAMMALS

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Since 1854, when the enumeration of the blood corpuscles was first successfully accomplished by Vierordt and Welcker, an enormous number of determinations have been made on the human subject both in health and disease, and the number of erythrocytes per cmm. given by these observers as normally present (5,000,000 for men and 4,500,000 for women) may be taken as approximately correct. In the lower animals, on the other hand, apart from those more immediately related to the veterinary profession, comparatively little attention has been given to the subject, and the following observations, made by us in this laboratory within the last six months, may be of sufficient interest to justify their publication.

The subjects were all healthy normal animals kept in the laboratory animal-house and yard, or lent by a farmer in this vicinity for the occasion. As a rule, no anaesthetic was used, but in some cases (badger, woodchuck), immobilization was found to be necessary and then ether was given.

Both red and white corpuscles were enumerated but no differential counts of the whites were attempted. The Thoma-Zeiss haemocytometer was employed throughout with Hayem's solution as the diluting fluid for the reds (dilution 1 in 200) and $\frac{1}{4}$ per cent acetic acid, tinted with methyl violet, for the whites (dilution 1 in 10). One hundred squares were counted and in some cases one hundred and fifty, two hundred and four hundred.

The blood was usually obtained from a slit or puncture wound through the skin of the ear or inner side of thigh, previously shaved, cleaned and dried, and from the drop or pool that collected we each took a sample practically simultaneously. After diluting for the reds and mixing thoroughly, two other samples were taken, in the same way, for the

TABLE

ANIMAL	RED	OBVS.	WHITE	OBVS.	DATE	REMARKS
Dog I ♀	3,697,000	5	12,300	3	Mar. 2	5 days before parturition
Dog I ♀	4,495,000	7	10,580	3	Mar. 4	3 days before parturition
Dog I ♀	3,466,000	4	12,370	3	Mar. 9	2 days after parturition
Dog I ♀	4,912,000	2			Mar. 23	16 days after parturition
Dog I ♀	7,052,000	2	11,000	2	Aug. 5	151 days after parturition
Pup I	4,080,000	2	12,160	1	Aug. 10	3 days old
Pup II	4,777,680	2			Aug. 11	4 days old
Pup III	4,492,800	2			Aug. 11	4 days old
Pup IV	4,095,100	3	11,900	1	Aug. 11	4 days old
Pup V	3,334,900	2			Aug. 23	16 days old
Dog II ♂	6,597,000	4			Aug. 16	2 years old
Dog II ♂	6,710,400	2			Aug. 23	
Dog III ♀	5,662,400	2			Aug. 16	41 days before parturition
Dog III ♀	6,501,000	4			Aug. 23	34 days before parturition
Dog III ♀	6,593,600	2			Aug. 23	Resting
Dog III ♀	7,132,000	2			Aug. 23	Exercise 30 minutes.
Pup A ♂	5,176,000	2	22,800	1	May 1	4 days old
Pup B ♂	3,464,000	2	15,400	2	May 4	7 days old
Pup C ♂	4,872,000	2	19,200	2	May 4	7 days old
Dog IV ♂	6,951,400	2			Mar. 16	2 years old
Dog V ♀	6,716,000	2			Mar. 18	Not pregnant
Dog VI ♀	7,600,000	2			Mar. 18	Not pregnant
Cat I	9,628,000	2	9,000	1	Apr. 13	Adult
Cat II	9,664,000	3	20,600	2	May 6	Adult
Rabbit I ♀	6,344,000	2	18,000	1	Apr. 13	Adult
Rabbit II ♂	7,256,700	2	7,200	2	Apr. 20	Adult
Rabbit III			10,030	10	Apr. 22	Adult
Horse I ♂	7,580,000	2			Apr. 22	Adult gelding
Horse II ♂	8,208,000	2	8,600	2	Apr. 22	Adult gelding
Cow I	7,812,000	2			Apr. 22	Adult
Cow II	7,498,700	3	11,600	2	May 4	Adult
Sheep I ♀	10,994,000	2	5,200	2	May 6	Adult
Sheep II ♀	9,808,000	1	8,800	2	May 11	Adult
Sheep III ♀	10,260,000	2	11,600	1	May 11	Adult
Goat I ♀	11,748,000	2	6,600	2	June 17	Angora; suckling kid
Goat I ♀	11,220,000	2			July 21	Angora milch

TABLE—Continued

ANIMAL	RED	OBVS.	WHITE	OBVS.	DATE	REMARKS
Goat II ♀.....	19,760,000	2	8,000	2	June 17	Angora; suckling kid
Goat II ♀.....	16,970,000	2			July 21	Angora milch
Kid I ♂.....	19,376,000	2	12,250	2	June 18	76 days old
Kid II ♂.....	22,344,000	2	12,800	2	June 18	60 days old
Swine I ♀.....	8,120,000	2	12,500	2	May 11	Adult
Swine II ♀.....	7,600,000	2	10,600	3	May 13	Adult
Monkey ♀.....	6,212,000	2	5,200	2	June 18	Adult
Prairie-dog I ♂..	9,901,760	2	6,400	2	Mar. 30	Adult
Prairie-dog I ♂..	8,057,600	3	6,200	3	Apr. 10	11 days after operation
Prairie-dog II ♂..	9,926,900	2			Mar. 30	Adult
Prairie dog II ♂..	8,201,400	2	9,200	2	Apr. 13	14 days after operation
Prairie-dog III ♂	9,694,000	2	6,400	1	Mar. 30	Adult
Prairie-dog III ♂	8,452,600	2	7,800	2	Apr. 13	14 days after operation
Woodchuck I.....	7,748,000	2	14,000	1	July 8	Adult
Woodchuck II ♀	6,097,000	6	10,300	2	Aug. 5	Adult
Woodchuck III ♂	6,296,000	2	12,400	1	Aug. 5	Adult
Badger I ♀.....	13,777,400	4	15,710	2	Mar. 25	2 days before parturition
Badger I ♀.....	14,213,000	2	16,730	3	May 20	54 days after parturition
Badger II ♀.....	7,880,000	2	14,100	2	June 18	83 days old
Badger II ♀.....	11,440,000	2	10,650	2	Aug. 5	132 days old
Tortoise I.....	388,000	1	12,200	1	July 6	
Tortoise II.....	896,000	1	13,600	1	July 6	
Tortoise III.....	984,000	1	11,200	1	July 6	
Frog I.....	652,000	5			Apr. 22	
Frog II.....	736,000	1			Apr. 22	
Frog III.....	616,000	1			Apr. 22	
Frog IV.....	360,000	1	10,400	1	May 8	

whites, and set aside in the counting chamber until the red corpuscles had been enumerated, four haemocytometers being used for each set of observations. Previous to this work we had had considerable practice in blood counting and as a rule our independent enumerations did not differ by more than 2 per cent, frequently by much less; when the disagreement was serious both were discarded and fresh samples taken.

Our results are presented in the preceding table where the average numbers of red and white corpuscles are given for each set of observa-

tions and the dates on which these were made; e.g., in dog 1, on March 2, the average of 5 counts was 3,697,000 for the reds, and of 3 counts 12,300 for the whites, etc.

Dog I, 7 years old, gave birth to her eighth litter of pups on March 7. The counts just before and after parturition show an unusually low number of red cells which appear to increase as the puerperium advances. With regard to the effect of pregnancy and parturition on the red cells opinions differ. Cohnstein (1) found an average of 9,742,000 in 7 pregnant and 12,090,000 in 12 non-pregnant sheep. From the examination by Thompson (2) of 12 pregnant women at different stages of gestation, he concludes that there is a moderate increase in red cells early in pregnancy, a diminution in the middle months with an increase again to the normal number towards its termination. Burnett and Traum (3) found that the count remained low in the bitch for some weeks after parturition, and a similar statement is made regarding the human subject.

The effect of age on the number of corpuscles is interesting. It is generally stated that the number of red cells is greater in the newly born and less in adolescents than in adults, but the evidence for this statement is conflicting. In the young pups of both dog I and dog III (see table) the red cells are distinctly below the average number for the adult normal dog, whereas, in the kids of our two goats the conditions are reversed. The figure 22,344,000 obtained in kid II is a higher count for reds than we have been able to find recorded anywhere in the literature, for any species of animal. Storch (4), on the other hand, found the average number of reds in adult goats to be 14,569,000 and in kids 10,150,000; in adult sheep (ewes) 9,039,000, in lambs (1-14 days old) 8,833,000, and in lambs two months old 13,232,000; in adult horses 7,639,000 and in foals 9,340,000; in adult cattle 6,219,000 and in calves 8,523,000; in adult swine 8,045,000 and in pigs 4,923,000. According to Burnett and Traum (3) the average number for dogs is 5,967,950 per cmm., while puppies, from less than a day to 20 days old, have 3,992,000 to 4,134,000 per cmm. Hayem (5) gives the average for cats as 9,900,000 while for kittens, from 4 to 8 days old, it is 5,357,000.

The badger on which we made our observations was a fine healthy specimen obtained from the state of Kansas. Two days after the first series of counts was made it gave birth to a litter of one, a female. This last we succeeded in rearing and domesticating and on June 18, when it was about three months old, the red cells numbered 7,880,000

per cmm. as compared with 14,213,000 for the mother a month before; on August 5, however, they had increased to 11,440,000.

From the above records it will be seen that no general statement can be made, covering all animals, regarding the effect of age on the number of red cells found in the blood.

The number of individuals of each species examined by us is not sufficient on which to base any conclusions concerning the influence of sex on the number of red corpuscles present in the blood.

The effect of muscular work is seen in the case of dog III (see table) where thirty minutes' exercise, running up and downstairs, raised the count from 6,593,000 to 7,132,000.

The three prairie-dogs were used in the laboratory for brain experimentation, the blood being obtained from the extirpation wound made in the cerebral cortex when the skull was trephined, and again when the animals were killed, about a fortnight after the first operation. In every case the red count is distinctly lower after than before the operation.

We find, for all the species examined, that there is much greater variation, relatively, in the number of white corpuscles than in the number of reds, both amongst different individuals of the same species and in the same individual from time to time. In forty-seven ordinary street dogs, such as find their way into a laboratory, and judged from their general condition to be normal, Musser and Krumbhaar (6) found that the average number of red cells was 5,973,739 per cmm., the highest count being 7,760,000 and the lowest 4,630,000. The white corpuscles were enumerated in only twenty-four of these animals, the average count being 15,923 and the extremes 33,050 and 8,800. All our dogs, with the exception of No. 1, had been born and bred in the animal house attached to the laboratory and were about two years old at the time the blood was examined. For these six dogs, three males and three females (excluding the counts taken from No. I near parturition, and from No. III as the result of exercise), the average for the red cells was 6,709,300 with extremes 7,600,000 and 5,662,400.

SUMMARY

From 229 blood counts made on 48 animals of 14 different species the average number of red and white blood corpuscles, respectively, per cmm. for each species was found to be as follows: Dog, adult, red 6,709,300, white 11,000; dog (few days old), red 4,268,560, white

16,290; cat, 9,646,000 and 14,800; rabbit, 6,800,850 and 11,743; horse, 7,894,000 and 8,600; cow, 7,655,350 and 11,600; sheep, 10,354,000 and 8,533; goat (adult, angora), 14,974,500 and 7,300; goat (kid, angora), 20,860,000 and 12,525; swine, 7,860,000 and 11,550; monkey (*Cercopithecus callitrichus*), 6,212,000 and 5,200; prairie-dog (*Cynomys ludovicianus* (Ord.)), 9,840,880 and 6,400; woodchuck (*Marmotta monax*), 6,713,700 and 12,250; badger (*Taxidea taxus* (Schreber)) adult, 13,995,200 and 16,220; badger, three months old, 7,880,000 and 14,100; same badger, four and a half months old 11,440,000 and 10,650; turtle (*Chrysemys elegans*) 756,000 and 12,330; frog (*Rana esculanta*), 591,000 and 10,400.

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THE INFLUENCE OF OIL OF CHENOPODIUM ON INTESTINAL CONTRACTILITY

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The present communication is one of a series of studies on the pharmacology of oil of chenopodium which has been carried on in this laboratory at various times during recent years. Some of the results, already reported by the senior author and his collaborators^{1,2} indicate that it has toxic properties affecting various tissues and organs of the body. It was of interest, therefore, to inquire in what manner the intestines react in the presence of this substance since, as far as we know, this has never before been the subject of investigation. Moreover, the importance of a better understanding of its action on the intestine is emphasized by its extensive use in the therapeutics of ascarides and hookworm, for any modification in the motor functions of the gut is likely to influence absorption and thus affect the toxicity of the oil. This is especially important on account of the change in the intestinal mucosa produced by the parasites against which oil of chenopodium is employed. (See Osler's *Practice of Medicine*.)

The method introduced by Magnus³ and adopted later by a number of investigators was used in these experiments. The animals were deeply anesthetized with urethane, the abdominal viscera exposed and segments of equal length, usually about 1.5 to 4 cm. were removed from various parts of the intestines. One end was attached to a glass hook fixed in the center of a rubber stopper, fitting tightly in the lower end of a cylinder of 100 cc. capacity. The latter was filled with Locke's solution, through which was passed a continuous stream of oxygen. The other end of the segment was attached to a recording lever. Exposure and excessive handling of the gut was avoided as much as

¹ Salant and Nelson: This Journal, 1915, xxxvi, 440.

² Salant and Livingston: This Journal, 1915, xxxviii, 67.

³ Magnus: Arch. f. gesamt. Physiol., 1904, cii, 123.

possible, the marked irritability and irregular action following such treatment furnishing the indication for this precaution. The cylinders containing the isolated pieces of intestine were kept in a warm water-bath maintained at a uniform temperature by means of an electric bulb. The substances to be tested were kept at the same temperature as the fluid surrounding the intestinal segments and were added to the contents of the cylinders. This procedure was adopted in order to avoid changes of temperature and exposure of the tissues.

A preliminary period during which observation on the behavior of the intestine in Locke's solution was studied preceded the experimental period when the intestine was in contact with the substance under consideration. This was followed by an after period when Locke's solution alone was substituted for the one containing the drug, care being taken to drain this off previously and wash the cylinder a number of times with Locke's solution.

Experiments with oil of chenopodium were also carried out on the intact animal. Rabbits were placed on the holder under urethane anesthesia, the abdomen was shaved and the peristaltic waves recorded by means of hooks⁴ attached to the skin from which threads passed to recording levers. The oil of chenopodium was made up in the form of an emulsion with acacia and added to Locke's solution.

EXPERIMENTS ON RABBITS

When segments of the duodenum were placed in Locke's solution containing oil of chenopodium in the proportion of 1 : 10,000, or 1 : 5000, decreased frequency of rhythmic contractions was observed within one or two minutes. Later the amplitude also began to diminish, the decrease being progressive, contractility disappearing altogether in seven and one-half to fifteen minutes after exposure to the influence of the oil of chenopodium. In one experiment with 1 : 5000 oil of chenopodium, this occurred after four and a half minutes; in two others with 1 : 10,000 oil of chenopodium, contractility was but moderately decreased after being subjected to its influence for twenty minutes.

That the injury is apparently not permanent is shown by the fact that complete recovery may take place when the tissues are thoroughly washed with Locke's solution and allowed to remain in it for some time. Coincidentally with the decrease in force and sometimes also in fre-

⁴ We are indebted to Dr. Livingston of this laboratory for suggesting this method.

quency of the rhythmic contractions, there was a depression of tone which was sometimes gradual but not infrequently quite abrupt and very marked as shown in figures 1 and 2.

The jejunum seemed to be less resistant to the influence of oil in some experiments, though no marked differences in action were observed in most instances. The decrease of amplitude and depression of tone occurred in two and a half to five minutes, while complete disappearance of contractions took place in seven to fifteen minutes. When the intestinal segments were thoroughly washed, after being subjected to the influence of oil of chenopodium thirty to forty-five minutes and allowed to remain in Locke's solution alone, the rhythmic contractions became normal or improved considerably. A return and sometimes a rise of tone above normal was also observed during this period.

The activity of the ileum placed in 1:5000 oil of chenopodium in Locke's solution

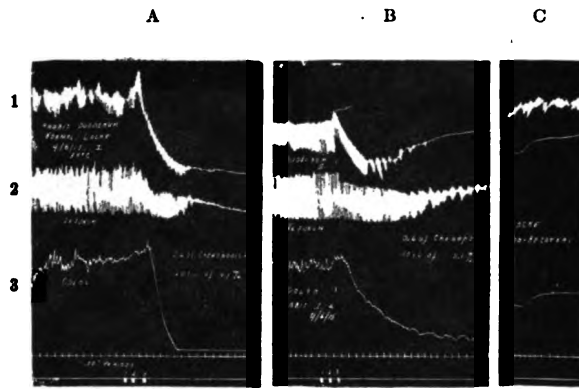


Fig. 1. Rabbit 1742. 1, Duodenum; 2, jejunum and 3, colon (A) subjected to oil of chenopodium 1/5,000. (B) Recovery in Locke solution and effect of 1/10,000 oil of chenopodium. (C) Returned to Locke solution alone.

continued for a variable time. The effect was slight at first, the amplitude as well as frequency being affected. At the end of fifteen to thirty-five minutes, contractility usually disappeared altogether. The tone was also depressed at this time but it was preceded, in some cases, by a primary rise soon after the oil of chenopodium was added to Locke's solution. Only slight improvement in contractility was observed when pure Locke's solution was substituted for oil of chenopodium after the usual treatment. The tone was frequently increased, and was in some cases even greater than in the fore period.

Segments of the colon suspended in Locke's solution containing oil of chenopodium in the proportion of 1:5000 manifested depression within one and one-half to four and one-half minutes after exposure. In

some cases complete paralysis was observed immediately after it was subjected to the influence of the oil; in others this was delayed as long as fifteen minutes but in most instances this occurred in two and one-half to five minutes. Although the effects were permanent in a majority of the experiments, contractility with a return of the normal tone might occur when the segments are placed in Locke's solution alone.

Tests were also carried out with 1 : 10,000 oil of chenopodium in Locke's solution. This had no effect on the contractility or tone in some cases, but depression was marked in a good many experiments.

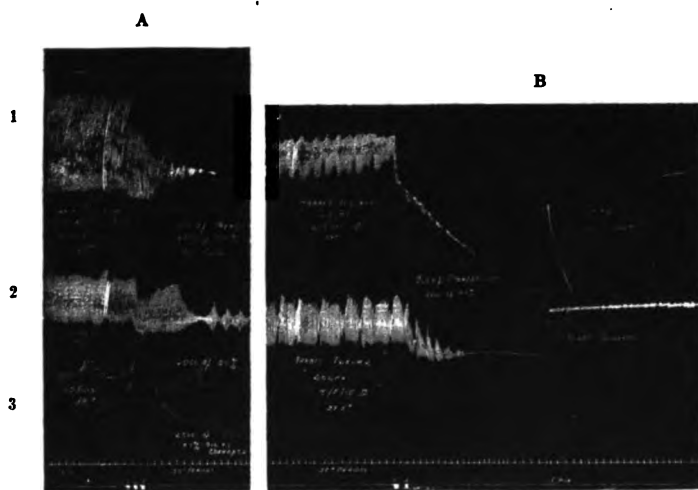


Fig. 2. Rabbit 1765. (A) 1, Jejunum; 2, ileum and 3, colon subjected to oil of chenopodium 1/5,000. (B) Recovery of ileum and jejunum in Locke solution showing effect of second treatment of oil of chenopodium 1/5,000 and recovery when returned to Locke.

It is worthy of remark that when the tissues had been previously subjected to the influence of a 1 : 5000 of the oil, depression of tone was complete, and nearly complete disappearance of rhythmic movements were always observed, even with 1 : 10,000 oil of chenopodium. Partial return of tone but no rhythmic contractions could be noticed when the segments of the gut were returned to Locke's solution alone, thus indicating cumulative effect.

EXPERIMENTS ON CARNIVORA

The response to oil of chenopodium was studied on the intestines of cats and one dog. As the isolated intestine of these animals is frequently apt to be less regular and more sluggish than that of the rabbit, the tests with oil of chenopodium were not quite as satisfactory. Its essential action could nevertheless be demonstrated also in these experiments. This is well shown in figures 3 and 4, and in the following abbreviated protocols which are typical of the results we obtained in a large number of experiments. It will be noticed that in some of

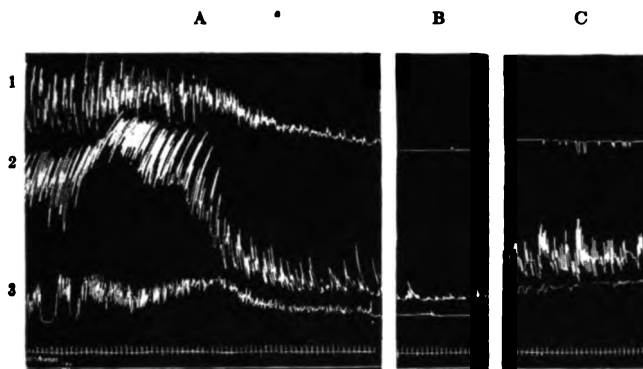


Fig. 3. Cat 394. 1, Duodenum; 2, jejunum and 3, ileum (A) subjected to oil of chenopodium 1/5,000. Curves show primary rise of tone followed by fall together with decrease of pendulum movements. (B) Shows disappearance of contractions in duodenum, weakness of contractions of jejunum and almost no contractions of the ileum. (C) Locke solution alone, showing slight recovery of duodenum and ileum with considerable improvement of the jejunum.

these experiments the influence of pilocarpine and barium chloride on the action of oil of chenopodium were also studied.

Cat 366. Segments of the duodenum, jejunum, and ileum were placed in oil of chenopodium 1 : 5000 for twenty minutes. Increased tone and frequency of pendulum contractions were the initial effects observed in all three portions of the intestine, the increase of tone being greatest in the ileum. Within six minutes depression of duodenal tone occurred and pendulum movements greatly decreased in force and frequency, disappearing altogether sixteen minutes after injection. Contractions and tone of jejunum were paralyzed. Pendulum movements of ileum became feeble and infrequent and disappeared in fifteen minutes, tone falling steadily during the time it was exposed to oil of chenopodium. No recovery of duodenum or of jejunum occurred in pure Locke's solution.

Cat 392. 1 : 10,000 oil of chenopodium in Locke's solution produced stimulation of pendulum movements of duodenal and jejunal segments and depression of pendulum movements of ileum, but the tone was increased. When the concentration of the oil of chenopodium was doubled, depression in all three segments was observed. Complete paralysis of pendulum movements as well as of tone followed with the increase of the concentration to 1 : 3333. Neither BaCl_2 1 : 500 nor 1 : 100,000 pilocarpine hydrochloride had any effect. No recovery was observed when pure Locke's solution was substituted for one containing oil of chenopodium.

Cat 390. Duodenum, jejunum and ileum placed in Locke's solution. Contractions strong. Pilocarpine hydrochloride was added, enough to make a con-

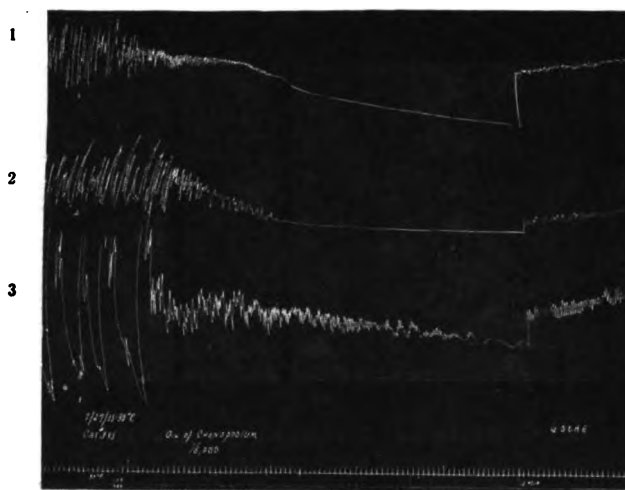


Fig. 4. Cat 395. Action of 1/5,000 oil of chenopodium upon longitudinal strips of 1, Duodenum; 2, jejunum and 3, ileum after cutting circular muscles. Gradual depression of muscular action until contractions disappeared. Partial recovery in Locke solution alone also shown.

centration of 1 : 100,000 caused powerful stimulation. Seven minutes later, 10 cc. 0.1 per cent oil of chenopodium was added, sufficient to make a concentration of 1 : 10,000. Contractions began to diminish appreciably five minutes later. Within fifteen minutes after adding oil of chenopodium, contractility of jejunum ceased. Duodenal segment and that of ileum were still active but contractions were feeble. Tone remained almost unchanged in all three segments. Barium chloride added, enough to make up a solution of 1 : 1000, slightly increased the tone of the duodenum and ileum and lowered somewhat that of the jejunum. Pendulum movements completely disappeared under its influence.

Cat 394. Oil of chenopodium in Locke's solution 1 : 5000. A steady decrease of tone reaching a maximum at the end of thirty minutes was observed. Rhyth-

mic contractions increased in frequency at first, disappearing completely about one hour after being in contact with oil of chenopodium in duodenum and ileum while they were still distinct though feeble in the jejunum. When changed to Locke's solution without chenopodium, contractility returned in the jejunum, the amplitude and frequency being nearly normal. Only slight improvement was observed in duodenum and ileum.

Dog 228. Duodenum, jejunum and ileum in 1 : 5000 oil of chenopodium in Locke's solution. Increase of tone set in immediately after oil of chenopodium was added and continued to rise as long as the segments remained in the oil, which was twenty minutes. After a preliminary increase of the pendulum movements there was a steady decrease which disappeared altogether in the duodenal segments in five minutes. Jejunum and ileum became barely perceptible at this time and continued without change as long as it remained in contact with the oil. When Locke's solution alone was substituted for Locke chenopodium, contractility of duodenal and jejunal segments almost completely revived.

THE INFLUENCE OF DRUGS ON THE ACTION OF OIL OF CHENOPODIUM

Experiments on rabbits. Barium chloride, pilocarpine hydrochloride, and caffeine, all of which are powerful stimulants, were tested for their antagonistic effect on oil of chenopodium. In some experiments they were added to Locke's solution before in others after, the administration of the oil. As shown in figure 6, barium chloride in a concentration of 1 : 3333 added before oil of chenopodium had little effect on its subsequent action. The tone was decreased and the rhythmic movements ceased soon after the intestinal segments came in contact with the oil. It may be remarked that although the decrease in tone was very pronounced, it was not below the normal height in the jejunum and ileum while it was distinctly greater in the duodenum than in the fore period. The reaction to barium chloride in the presence of oil of chenopodium, although considerably diminished, was nevertheless quite distinct when the concentration of the oil was in the proportion of 1 : 10,000. The rise of tone

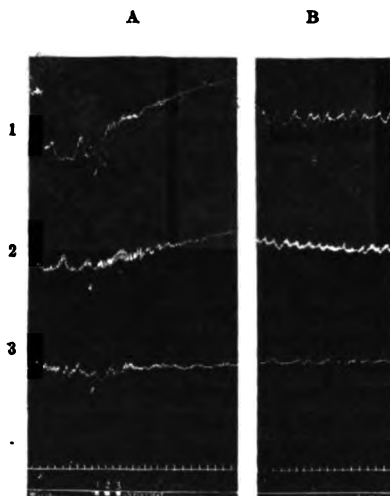


Fig. 5. Dog 228. 1, Duodenum; 2, jejunum; 3, ileum. (A) Showing effect of oil of chenopodium 1/5,000. (B) Recovery in Locke solution without oil.

was abrupt, remaining permanent in some cases but in most instances a gradual descent occurred. In the presence of 1 : 5000 oil of chenopodium, the same concentration of barium chloride (1 : 1000) failed to produce any appreciable rise of tone. The reaction likewise varied with the length of time during which it had been subjected to the influence of the oil previous to treatment with barium chloride. While the increase of tone was quite marked, when barium chloride was added ten to fourteen minutes after the intestinal segments had been under the influence of oil of chenopodium, a longer period of contact with it—thirty-

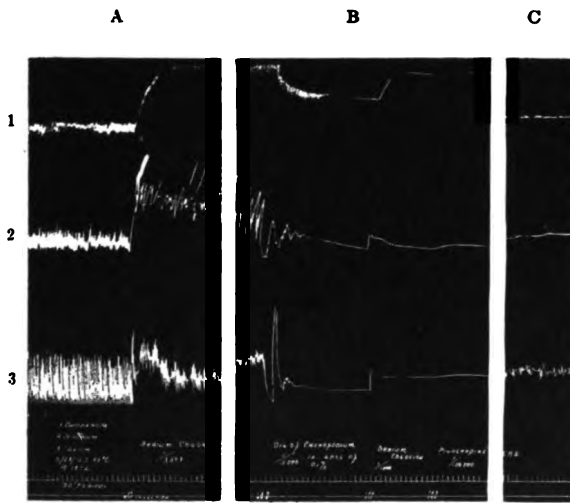


Fig. 6. Rabbit 1872. 1, Duodenum; 2, jejunum and 3, ileum. (A) Barium chloride 1/3,333. (B) Followed by oil of chenopodium 1/5,000. Barium chloride 1/1,000 caused rise of tone but slight reaction to pilocarpine 1/100,000. (C) Returned to Locke solution alone. Slight recovery of duodenum and ileum. No change in jejunum.

five to forty-five minutes, produced a much weaker response to barium chloride.

The reaction to pilocarpine in various concentrations was usually negative, though in one instance the tone was increased. It may be added that barium chloride and pilocarpine were tried in the same experiment. It was found that the barium effect could be elicited when the tissues no longer responded to

pilocarpine (see figure 6), which was mixed with the solution, either before or after barium chloride was added to Locke's chenopodium.

Caffeine in the proportion of 1 : 5000 and 1 : 2000, produced a very marked stimulation of the contractility of the untreated intestine. The effect was different, however, when employed in the same concentrations in the presence of oil of chenopodium, though only used in the proportion of 1 : 10,000. Transitory increase of tone of the duodenum and depression of tone of the ileum, which was permanent in some cases

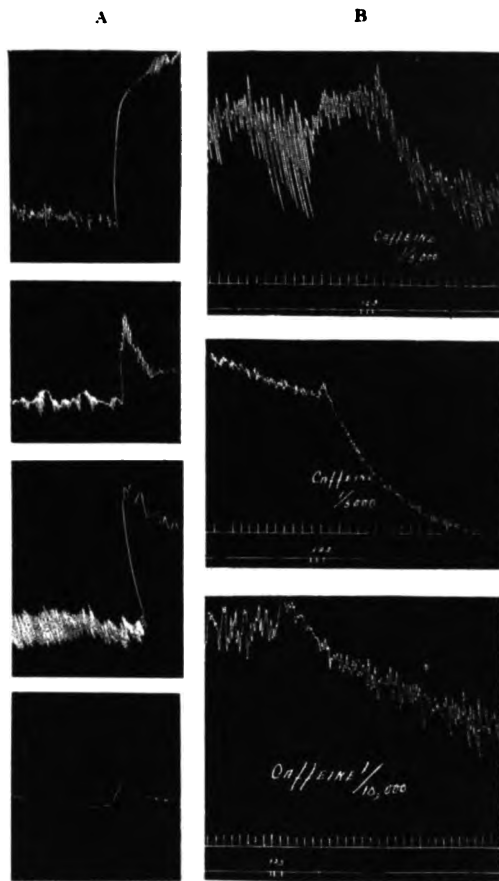


Fig. 7. Effect of 1/5,000 caffeine after subjection of intestine of rabbit to oil of chenopodium 1/10,000. (A) Segments of duodenum. (B) Segments of ileum.

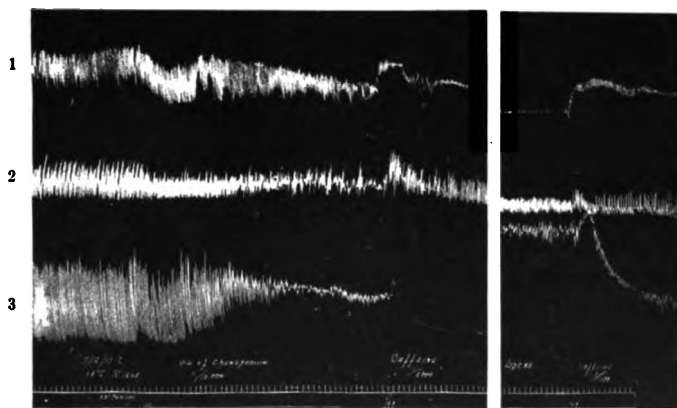


Fig. 8. Rabbit 1868. 1, Duodenum; 2, jejunum and 3, ileum (A) treated with oil of chenopodium 1/10,000 followed by caffeine 1/5,000. (B) shows improvement in Locke solution and effect of 1/5,000 caffeine.

as shown in figure 7, marked this treatment. In some experiments its addition to oil of chenopodium was without any effect. In others, however, this caused occasional stimulation of rhythmic movements. When returned to pure Locke's solution no recovery, or only a moderate degree of activity, could be observed. In one experiment caffeine produced a very pronounced depression of tone in the after period, which suggests that the effect of the oil may persist even after the surviving intestine has been thoroughly washed.

EXPERIMENTS ON CATS

The reaction to barium chloride and pilocarpine was likewise profoundly modified in the intestine of the cat by oil of chenopodium. Little or no response was obtained with barium chloride solutions of 1 : 1000 when the intestinal segments have been under the influence of the oil in concentrations of 1 : 3333 and 1 : 5000. A noticeable increase in tone was observed, however, in one instance when the concentration of barium chloride was increased to 1 : 500 in a dilution of 1 : 5000 oil of chenopodium (Cat 389). The test with pilocarpine was entirely negative in one experiment while a temporary stimulation was observed in another in which the concentration of oil of chenopodium present amounted to 1 : 3333. It may be remarked that in this instance barium chloride, which was added after pilocarpine, produced a very good reaction in the ileum, none in the jejunum, and a mild transitory effect only in the duodenum.

INFLUENCE OF OIL OF CHENOPODIUM ON PERISTALSIS

The movements of the intestines under the influence of oil of chenopodium were also studied in the intact animal. Rabbits under urethane anesthesia were employed for this purpose. This method was found to be particularly well adapted for the study of peristalsis in the living animal since the waves of contractions, especially those of the caecum, appeared in most animals with remarkable distinctness and regularity. A 2 per cent emulsion of oil of chenopodium in olive oil and a small amount of acacia was injected into the ear vein and its effect recorded as stated above. Special attention was paid to size of dose as well as to the influence of repeated dosage. Experiments were also conducted for testing the action of caffeine when given either before or after oil of chenopodium was introduced into the circulation. As a control we used intravenous injections of a solution made up with acacia and coconut oil in Locke's solution consisting of the same proportion but without oil of chenopodium. Only a few of these,

however, were made but a much larger number of experiments were carried out with the oil of chenopodium. A detailed description of some of these is contained in the following records, which are typical of the action produced.

April 9, 1915. Rabbit No. 1756, brown and mole color, female. Weight, 1500
Mixed diet. Never before under experimentation. Abdomen shaved.

10.30 a.m. 2 grams of urethane.

2.15 p.m. Peristalsis active.

2.28 p.m. Wave.

2.30 p.m. Wave.

2.33 p.m. Wave.

2.34 p.m. Wave.

2.35 p.m. Wave.

2.48 p.m. 10 cc. of 2 per cent of oil of chenopodium injected into the ear vein. Thirty seconds later the veins of the ear were very much dilated.

3.05 p.m. No true peristalsis though slight peristaltic waves are seen above the points of attachment.

3.25 p.m. No peristalsis. Respiration shallow with occasional deep breaths. Injection of 80 mgs. of sodium citrate into the ear vein. No peristalsis.

4.00 p.m. No peristalsis. Gut exposed. Oil found in the subcutaneous tissue over the abdomen. Point of contractions mentioned proved to be small intestine.

March 27, 1915. Rabbit No. 1787.

Weight, 1740. Male, Belgian. Mixed diet. Never before under experimentation. Control.

10.00 a.m. 2 grams of urethane.

2.40 p.m. Rapid peristalsis.

2.42 p.m. Injection of 10 cc. of control solution (1 per cent of oil of cocoanut, 0.1 per cent acacia in Locke) in ear vein. No cessation of peristalsis.

2.44 p.m. 10 cc. of control solution in the ear vein.

2.45 p.m. Faint wave.

2.47 p.m. Wave, strong and rapid. Continuous peristalsis.

3.00 p.m. Peristalsis present.

March 27, 1915. Rabbit 1786. Weight, 1310. Male. Black. Mixed diet. Never before under experimentation. Abdomen shaved.

10.00 a.m. 2 grams urethane.

11.00 a.m. Peristalsis active appearing every 40 seconds, strong and rapid.

11.05 a.m. Peristalsis active. Injection of 2.5 cc. of a 2 per cent oil of chenopodium emulsion into the veins of the ear.

11.09 a.m. Three waves passed. Wave in rapid propagation. Waves appear in following periods:

40 seconds.

30 seconds.

40 seconds.

50 seconds.

50 seconds.

11.15 a.m. 7.5 cc. of chenopodium emulsion. Peristalsis depressed. No wave for two and one-half minutes.

11.20 a.m. No peristalsis.

11.30 a.m. No peristalsis.

11.35 a.m. No peristalsis. Upon mechanical stimulation small and slow waves pass over caecum.

11.46 a.m. Faint, slight questionable peristalsis seen at the "Point of Origin" of waves. Perhaps this was the ileum or ileo caecal junction.

12.15 p.m. Slight waves seen.

1.15 p.m. No contractions seen.

2.30 p.m. No contractions seen.

April 2, 1915. Animal weight 1390, apparently normal. Returned to "Runs" and given mixed diet.

March 23, 1915. Rabbit 1712. Weight, 1680. Male. Belgian. Mixed diet. Under experimentation February 9 to March 9.

10.00 a.m. 2 grams of urethane. Abdomen shaved.

3.30 p.m. 4 cc. of 2 per cent oil of chenopodium, 1 per cent oil of cocoanut, and 0.8 per cent acacia in Locke. Injected into ear vein. Peristalsis slightly increased.

3.35 p.m. 5 cc. of the above emulsion given by intravenous injection. Peristalsis decreased in frequency and rate of propagation.

3.46 p.m. 4 cc. of the emulsion injected intravenously. Complete inhibition of peristalsis.

3.56 p.m. Weak contraction just discernible.

4.03 p.m. Mechanical stimulus applied to caecum followed by slight contraction. Repeated stimulation was followed by no contraction.

4.15 p.m. Complete inhibition. Animal placed in isolated cage.

March 27. Normal, returned to "Runs."

April 9, 1915. Rabbit No. 1704. Weight, 1645. Male. Belgian. Mixed diet. Never before under experimentation. Abdomen shaved.

10.45 a.m. 2 grams of urethane.

12.00 m. Peristalsis marked, strong, and rate of propagation rapid.

12.19 p.m. 3 cc. of 2 per cent oil of chenopodium emulsion injected into the ear vein.

11.20 p.m. 4.5 cc. of the above emulsion injected. Followed by depression in the rate of occurrence of peristaltic waves.

12.25 p.m. Three waves since injection.

12.26 p.m. Wave.

12.27 p.m. Wave.

12.28 p.m. Wave.

12.29 p.m. Wave.

12.31 p.m. Wave.

12.33 p.m. Wave.

12.34 p.m. Wave.

12.35 p.m. Wave.

12.39-40 p.m. Injection of 5 cc. of 2 per cent oil of chenopodium. Peristalsis seen in small intestine.

12.42 p.m. No peristalsis in caecum. Some movements in the small intestine, though irregular in frequency.

12.48 p.m. No peristalsis in the caecum; contractions in the small intestine.

1.15 p.m. As yet no peristalsis in the caecum. None seen in the small intestine.

1.20 p.m. No peristalsis, abdomen flat. Animal discarded.

April 12, 1915. Animal up, not depressed and apparently normal. Returned to the "Runs."

DISCUSSION

Whatever the mechanism of the action of oil of chenopodium on the intestine, the results of the experiments presented in this investigation indicate, as in previous studies with this substance, that it is a powerful depressant also of the intestine. The inhibitory effect, however, may be temporary, lasting as long as the tissues are in contact with the oil for if they are thoroughly washed with and then allowed to remain in Locke's solution, recovery varying in degree may occur even after it has been subjected to the action of high concentrations of the oil. The reaction varied in different portions of the intestine, the effect being more marked in the ileum but was much more pronounced in the colon than in the duodenum or jejunum.

That the action is more effective in this case is also shown by the failure of the colon to resume its normal activity when it was surrounded again by pure Locke's solution, as contractility was much weaker or altogether absent.

The work of Weiland⁵ is worthy of notice in this connection as it suggests the presence of a substance in the intestine which is antagonistic to oil of chenopodium. It may be recalled that he obtained an extract from the mucosa as well as from the muscular coat which caused powerful stimulation of the movements of the small intestine, but had little or no effect on the large intestine. The failure of this substance to react may account perhaps for the different behavior of the colon from that of the small intestine when treated with oil of chenopodium.

The results on the influence of drugs are of particular interest as regards the mechanism of the action of oil of chenopodium. The stimulating effect of barium, pilocarpine and caffeine was permanently abolished or diminished. In no case did any of these drugs exert any appreciable antagonistic action. Indeed, we observed that caffeine had, on the contrary, a decided tendency to cause sometimes further

⁵ Weiland: Arch. f. gesamt. Physiol., 1912, cxlvii, 171.

decrease of tone. Dixon's⁶ observations may be recalled in this connection. He found that a strong solution of lactic acid applied externally to the frog's stomach induced contraction followed by gradual relaxation, but when this treatment was employed during the stage of relaxation further depression resulted. The negative results with pilocarpine might indicate that nerve ends as well as muscle fibers are paralyzed, but as stated above, and as shown in figure 6, the response to this drug failed while barium still produced a well marked reaction, thus showing paralysis of the nerve ends. That the muscle fiber is also involved is made apparent by its mode of action in the presence of barium. Although the reaction to barium after subjecting the intestine to the influence of 1 : 10,000 oil of chenopodium was very distinct, it was inferior to that of the normal intestine while it completely disappeared in higher concentrations of the oil. The action of oil of chenopodium is therefore exerted on the nerve ends as well as on the muscular structures, but the evidence brought forward points to the greater resistance of the latter.

The test with oil of chenopodium when injected intravenously may be regarded as corroborative of the results obtained with the isolated intestine. The effect varied with the amounts introduced. The smallest dose given, 0.045 cc. of the oil per kilo produced in one experiment a slight increase of peristalsis, in another case 0.045 cc. per kilo arrested movements of the caecum for four minutes. Medium doses 0.09 to 0.1 cc. per kilo invariably decreased the frequency of peristalsis but total suspension of activity was not noticed after such amounts. This was obtained when the dose exceeded 0.12 cc. per kilo (figure 9), producing in one experiment complete arrest of movements of the caecum for seventy-two minutes. On the other hand, a larger dose produced, in one experiment, decreased frequency only. The data at hand indicate, however, that 0.15 to 0.2 cc. per kilo inhibit peristaltic action in the caecum for a considerable length of time. Only a few observations were made on the small intestine. Well marked contractions were seen after a large dose of oil of chenopodium, while there was complete arrest of movements of the caecum. The large amount of oil of chenopodium required to depress movements in the caecum of the living animal as observed in these experiments deserves explanation, especially since it has been shown by Salant and Livingston⁷ that a fall of blood pressure and a marked decrease of the volume of the kidney

⁶ Dixon: *Journal of Physiology*, 1902, xxviii, 57.

⁷ Salant and Livingston: *l. c.*

followed its intravenous administration. That a close relation exists between the blood supply of the intestines and their motor functions has been maintained by a number of investigators. According to van Braam Houckgeest,⁸ Pal,⁹ and others, peristalsis varies with the volume of blood in the vessels of the digestive tract, an increased flow causing stimulation while anemia decreases, or if very severe, may abolish intestinal movements. Paralysis of the vagus, observed by Salant and Livingston, is another factor to be taken into consideration. That it may contribute materially toward the direct action of the oil of chenopodium on the intestine also appears from the results of Meltzer and Auer¹⁰ who found that the elimination of the stimulating effect of the

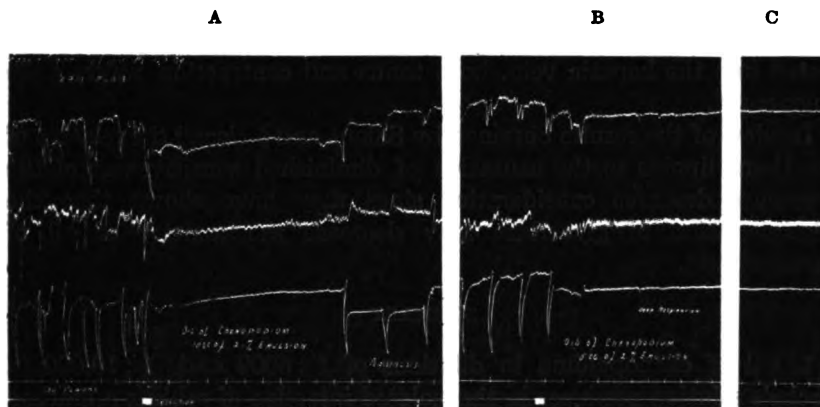


Fig. 9. Rabbit 1777. Peristalsis of caecum in intact animal. 10 cc. of 2 per cent emulsion of oil of chenopodium injected into the ear vein. (A) Movement of caecum after six minutes. (B) 5 cc. more of the emulsion 2 per cent oil of chenopodium injected into the ear vein. No peristalsis followed as seen in (C).

vagus is followed by cessation of caecal peristalsis. Inhibition, partial or complete, of caecal movements also follow, therefore, the introduction of oil of chenopodium into the circulation. Its action on peristalsis when injected intravenously is thus aided, by its effect on the circulation as well as by its depression of the motor nerve of the intestine. That large doses are nevertheless required to produce depression of its activity may be due in the first place to rapid destruction or elimination, but we have seen that this is not probable. There is, on the con-

⁸ van Braam Houckgeest, Arch. f. gesamt. Physiol., 1872, vi, 266.

⁹ Pal: Wiener Med. Presse, 1901, iv, 2017.

¹⁰ Meltzer and Auer: Proc. Soc. Exper. Biol. and Med., 1906, iv, 37.

trary, a marked tendency to cumulation both in the isolated intestine as well as in the intact animal. The explanation may be found perhaps in the presence of neutralizing or antagonistic substances in the tissues of the body. The following observations indeed lend support to this view. Ott¹¹ stated that the intravenous infusion of an aqueous extract of the spleen stimulates peristalsis while splenectomy is followed by diminished contractility which may be restored to its normal condition or even increased by the extract. The observations of Enriquez and Hallion,¹² and later of Weiland¹³ on extracts of spleen and other organs are in agreement with those of Ott. Dixon's¹⁴ findings with pilocarpine in experiments on the frog's stomach are also suggestive in this connection for he noticed that its external application increased the amplitude of contraction waves but had little effect on tone. When injected into the hepatic vein, both tonus and contraction waves were augmented.

In view of the results obtained by Salant and Nelson¹⁵ the relation of the tissue lipoids to the causation of diminished sensitiveness of the intestines deserves consideration since they have shown that the administration of glycerides may decrease the toxicity of oil of chenopodium.

SUMMARY

1. Oil of chenopodium in dilutions of 1 : 5000 and 1 : 10,000 in Locke's solution produces in the isolated intestine of rabbits a marked decrease of tone which remains permanent and diminishes frequency as well as force of contractions which disappeared altogether in twenty to twenty-five minutes. Recovery occurred when the intestinal segments were placed in Locke's solution without oil of chenopodium.

2. In carnivorous animals, oil of chenopodium usually, but not always, causes a preliminary rise of tone followed by a steady decline. Rhythmic contractions may increase in frequency but disappear finally. Recovery may take place when the segments are put into Locke's solution.

3. The reaction to oil of chenopodium was greater in the ileum than in the duodenum or jejunum, but was most marked in the colon.

¹¹ Ott: Medical Bulletin, 1897, 376.

¹² Enriquez and Hallion: Compt. Rend. Soc. d. Biol., 1911, lxxi, 488.

¹³ Weiland: l. c.

¹⁴ Dixon: l. c.

¹⁵ Salant and Nelson: l. c.

4. Caffeine has no antagonistic effect but may, on the contrary, aid depression of tone caused by oil of chenopodium.

5. Neither barium chloride nor pilocarpine has a true antagonistic effect but may prevent to a small extent depression of tone when added before oil of chenopodium. Pilocarpine has no action on intestine which has been poisoned by oil of chenopodium, but barium produces an increase of tone.

6. Nerve ends as well as muscle fiber are attacked by oil of chenopodium, but the latter is more resistant.

7. Relatively large doses of oil of chenopodium are required to inhibit peristalsis in intact rabbits by intravenous injection. The presence of substances antagonistic to oil of chenopodium is offered as an explanation.

THE INFLUENCE OF STIMULATION OF THE DEPRESSOR NERVE UPON SUPRARENAL SECRETION

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During recent years much evidence relating to the secretory innervation of the suprarenal glands has accumulated. Dreyer¹ was the first to show that blood, collected from the suprarenal vein of a dog during stimulation of the splanchnic nerve possessed the power of increasing the blood pressure when injected into the vein of another dog to a greater degree than did blood similarly collected before stimulation. Ample confirmation of this result is to be found in the work of Asher,² Tscheboksaroff,³ Elliott,⁴ von Anrep⁵ and many others, and it is now regarded as established that secretory fibers for the suprarenal glands are contained in the splanchnic nerves. Elliott's work has further shown that the center of these fibers is situated in the medulla in close association with the vasomotor center.⁶ Like the latter, it may be stimulated directly as a result of asphyxia,⁷ reflexly by excitation of sensory nerves,⁸ and by impulses reaching it from higher centers (mechanical injury,⁹ fear, rage,¹⁰ etc.). The nervous mechan-

¹ Dreyer: American Journal of Physiology, 1898-1899, ii, 203.

² Asher: Zentralblatt für Physiologie, 1910, xxiv, 927; Zeitschrift für Biologie, 1912, lviii, 274.

³ Tscheboksaroff: Archiv für die gesamte Physiologie, 1910, cxxxvii, 59.

⁴ Elliott: Journal of Physiology, 1912, xlv, 374.

⁵ von Anrep: Journal of Physiology, 1912-1913, xlv, 307.

⁶ Elliott: Loc. cit., p. 406.

⁷ Cannon and Hoskins: American Journal of Physiology, 1911, xxix, 274. Starkenstein: Zeitschrift für experimentelle Pathologie und Therapie, 1911, x, 95. Czubalski: Zentralblatt für Physiologie, 1913, xxvii, 580.

⁸ Cannon and Hoskins: American Journal of Physiology, 1911, xxix, 274. Elliott: Loc. cit., p. 406.

⁹ Elliott: Loc. cit., p. 385.

¹⁰ Cannon and de la Paz: American Journal of Physiology, 1911, xxviii, 64.

ism of secretion may be centrally excited by ether,¹¹ chloroform,¹¹ morphine,¹¹ strophanthin¹² and digitoxin¹² and peripherally by nicotine¹³ and pilocarpine.¹⁴

It is noteworthy that the experiments which form the basis for the above statements yield no evidence of the existence of a nervous mechanism for inhibiting suprarenal secretion other than that which is implied in cessation or absence of stimulation, exhaustion of the glands, or alterations in blood flow through the glands. Having in mind the close relationship between the centers for suprarenal secretion and the vasoconstrictor mechanism as emphasized by Elliott and von Anrep, the thought occurred to us that inhibition of suprarenal secretion might be produced by influences known to inhibit the vasoconstrictor center. The most clean cut and constant example of inhibition of the vasoconstrictor center is to be found in its reaction to stimulation of the depressor nerve. The experiments described in this paper were therefore designed to answer the question, "Does stimulation of the depressor nerve result in a decrease in the rate of discharge of epinephrine from the suprarenal gland: if so, can it be attributed to an influence exerted upon the secreting structures rather than to effects upon blood flow through the gland?" We believe that evidence has been secured which permits an affirmative answer to both parts of the question.

TECHNIQUE

The experiments were performed on rabbits, prepared for collection of blood from the suprarenal veins according to Dale's description of Biedl's method.¹⁵ They were narcotized by introduction of 1.8-2.0 grams of ethyl urethane per kilo into the stomach: a little ether was given by inhalation during the operation: no ether was necessary during the experimental period. The carotid artery, left external jugular vein, vagus and depressor nerves were exposed. A cannula was inserted into the carotid artery for recording blood pressure, and

¹¹ Elliott: Loc. cit., p. 383.

¹² Richards and Wood: *Journal of Pharmacology and Experimental Therapeutics*, 1915, vi, 283.

¹³ Cannon, Aub and Binger: *Journal of Pharmacology and Experimental Therapeutics*, 1912, iii, 379.

¹⁴ Dale and Laidlaw: *Journal of Physiology*, 1912-1913, xlv, 1.

¹⁵ Dale and Laidlaw: *Journal of Physiology*, 1912-1913, xlv, 1. Biedl: *Archiv für die gesammte Physiologie*, 1897, xlvii, 481.

into the peripheral stump of left external jugular vein. Both vagus nerves were cut. The depressors were identified by the results of electrical stimulation. In some experiments the left median nerve was prepared for central stimulation.

The abdomen was opened by a long median incision, the coeliac axis, superior and inferior mesenteric arteries ligated and the animal eviscerated. The renal arteries and veins and the spermatic (or ovarian) veins were tied. The abdominal aorta was ligated just below the renal arteries. After ligating the inferior vena cava a cannula was inserted into its central stump just below the entrance of the renal veins. A loose ligature was placed about the inferior vena cava above the entrance of the right suprarenal vein. Fifty mgm. of hirudin per kilo, dissolved in salt solution, were injected into the vena cava after the operation was completed and before any samples of blood were taken. The temperature of the animal was maintained throughout the experiment by an electric heating pad. In some experiments artificial respiration was maintained by a Meyer pump; in others, natural respiration was adequate.

For the purpose of estimating epinephrine output of the glands blood was drawn from the suprarenal veins by tightening the ligature about the inferior cava above the suprarenal veins and simultaneously opening the clamp on the cava below the suprarenal veins. In experiments 1, 2, 3 and 5 the blood was collected in a pipette temporarily attached to the cannula in the cava by a short piece of rubber tubing; in the others, the cannula in the cava was made from a 2 cc. graduated pipette; the neck of the cannula was made as close as possible to the zero mark and a side tube, by which the pipette could be emptied, was sealed in at that point. In experiments 5-11 we endeavored to make the duration of collection of blood samples from the suprarenal veins the same for each sample by placing a finger over the open end of the pipette and retarding to a greater or less extent the flow of blood into it. The purpose was to make the rate of flow of blood through the glands constant. It seems hardly possible that this purpose was adequately accomplished on account of the distensibility of the walls of the veins in the space bounded by the gland capillaries, loose ligature and cannula. In the other experiments, therefore, the blood was allowed to flow freely, the duration of the period of collection being recorded on the kymograph by an electric signal and the volume of blood accurately measured.

Stimulation of the depressor was produced by an interrupted current of moderate strength which could be borne by the tongue without discomfort. In collecting blood during depressor stimulation, the stimulus was applied 15-30 seconds before collection begun and was maintained until it was finished.

In experiments 1-16 the lumbar divisions of the suprarenal veins were not ligated. In experiment 17, this was done on the left side; in experiment 18, the lumbar divisions of both suprarenal veins were completely ligated close to the gland and we therefore believe that in this experiment blood was secured from the suprarenal veins exclusively. The agreement of the results of experiments 18 and 17 with those of the earlier experiments leads to the conclusion that mixture of suprarenal blood with that from other sources has not been a factor of importance in the results obtained.

The epinephrine content of various samples of blood was tested by means of the isolated intestinal muscle method as employed by Cannon and de la Paz.¹⁶ The muscle used was excised from the intestine of a deeply chloroformed cat,¹⁷ prepared in a dish of oxygenated Ringer's solution at 37°C. and mounted in Ringer's solution in a muscle chamber of about 3 cc. capacity so that its contractions could be recorded. A slow current of oxygen continually bubbled through the fluid in the chamber. The chamber was immersed in a large water bath, in which were suspended also the tubes containing blood samples to be tested. Changes in muscular contraction due to temperature variations were, we believe, excluded.

Each sample of blood was oxygenated for two or three minutes before being tested.

As a routine, the intestinal strip was allowed to remain in Ringer's solution until its contractions became reasonably uniform. Then control blood—that drawn from peripheral stump of the external jugular or, in experiments 1 and 2, from the inferior cava—was substituted. When the muscle contractions in this medium had become uniform, the samples from the suprarenal veins were tested in the order in which they were drawn. Between each two tests of suprarenal blood, the muscle was immersed in control blood. In the later experiments of the series all blood samples were accurately diluted with Ringer's solution, made up without bicarbonate.

¹⁶ Cannon and de la Paz: *American Journal of Physiology*, 1911, xxviii, 64.

¹⁷ In our experience the cat's intestinal muscle has proved to yield more active, stable and uniform preparation than that of the rabbit.

RESULTS

The abbreviated protocols of nine experiments are given at the end of the paper. They agree in showing that the intensity of the reaction for epinephrine in blood collected from the suprarenal veins is lessened if collection of the blood is made during stimulation of the depressor nerve. The degree of this effect is variable. In experiment 2 the blood collected during depressor stimulation caused no inhibition of the intestinal strip, whereas blood collected five minutes later under identical conditions save that the depressor nerve was not stimulated caused complete inhibition of both tonus and rhythmic contractions. In other experiments the difference is apparently slight; the suprarenal blood taken when the depressor nerve was not stimulated gave complete inhibition of tonus and contractions while that similarly collected during stimulation of the depressor gave inhibition of tonus with only partial inhibition of contractions. On cursory examination of the record, such a difference as this between two samples of blood might appear to be too slight to be worthy of serious consideration. It is to be remembered, however, that the maximal reaction of the intestinal muscle to epinephrine is complete inhibition: obviously the method, without modification, gives no means of recognizing whether a sample of blood contains more than the minimal amount of epinephrine sufficient to give the maximal reaction. Evidence in this connection is found in the record of experiments 14, 17 and 18. In experiment 14 (fig. 7) blood sample 5 taken during depressor stimulation is compared with samples 4 and 6 taken in the absence of depressor stimulation. When first tested, 4 and 6 gave complete inhibition, 5 showed inhibition of tonus which was interrupted by rhythmic contractions. All three samples were allowed to stand for about twenty minutes under identical conditions. When tested the second time, 4 and 6 yielded a reaction which was just short of maximal, while sample 5 yielded a result which could hardly be called a positive reaction. The spontaneous disappearance of epinephrine from the three samples was just sufficient to reveal the marked excess of that substance in samples 4 and 6 over sample 5. Similarly in experiment 17 (fig. 8) the first comparison of samples 4 and 5 showed no difference in intensity of reaction. Further dilution and standing (4a and 5a) showed that sample 5 which was collected during stimulation of the depressor, contained decidedly less epinephrine than sample 4. Again in experiment 18 (fig. 9) successive dilution showed the great disparity in epinephrine content of blood samples 5 and 6.

It may be well to point out that the order in which blood samples were taken was not uniform and hence the observed effect of depressor stimulation cannot be explained as the result of a constant source of error such as loss of blood involved in collection of samples. In experiments 1, 11, 13 and 14 the collection of blood during depressor stimulation was both preceded and followed by the collection of a sample during no nerve stimulation. In the first part of experiment 3, the order of collection was reversed in the later as compared with the earlier part of the experiment.

In two experiments (13 and 14) an attempt was made to increase the normal output of epinephrine by stimulation of a sensory (median) nerve, with the idea that by such a procedure the decrease caused by depressor stimulation might be more striking. The results appear, however, to be of the same order as those in which this trial was not made.

In one experiment evidence of a somewhat different sort is to be found in support of the conclusion that the epinephrine content of the suprarenal blood can be lessened by central stimulation of the depressor nerve. It is known that section of the depressor nerve is not commonly followed by rise in arterial pressure. Bayliss¹⁸ showed, however, that if plethora were induced by infusion of salt solution section of the depressors caused marked rise of pressure. These facts support the view that while the depressor is not normally in a state of excitation it may be physiologically stimulated by plethora in such a way as to take part reflexly in the adjustment of the circulation to increased volume of fluid in the blood vessels. In experiment 17, immediately after the intravenous infusion of 25 cc. of salt solution, blood sample 1 was taken from a suprarenal vein. Both depressor nerves were then cut and blood sample 2 was similarly taken as soon as possible. The reaction for epinephrine in sample 1 was negative; in sample 2, strongly positive. In this experiment section of the depressors caused a rise of arterial blood pressure of approximately 20 mm. of mercury.

In the experimental protocols at the end of this paper are included the rates of flow of blood from the suprarenal veins. On these figures is based our conclusion that the decrease in epinephrine content attendant upon stimulation of the depressor nerve is due to an effect upon secretory processes in the gland and is not the result merely of faster flow of blood through the gland. For convenience the figures, expressed

¹⁸ Bayliss: *Journal of Physiology*, 1893, xiv, 303.

as fractions of a cubic centimeter per second, are tabulated below. The figures in parentheses are the numbers of the blood samples.

Rates of blood flow from suprarenal veins

EXPERIMENT NO.	1	2	3		5	
Before depressor stim.	(B ₂) 0.272		(2) 0.136			
During depressor stim.	(B ₄) 0.091	(2) 0.091	(3) 0.111	(7) 0.077	(2) 0.091	(5) 0.115
After depressor stim....	(B ₆) 0.166	(3) 0.071		(8) 0.059	(3) 0.076	(6) 0.103
EXPERIMENT NO.	11	13		14	17	18
Before depressor stim...	(2) 0.06	(3) 0.083	(5) 0.055	(4) 0.05	(4) 0.08	(5) 0.05
During depressor stim.	(3) 0.058	(4) 0.09	(6) 0.059	(5) 0.066	(5) 0.07	(6) 0.057
After depressor stim....	(4) 0.029		(7) 0.045	(6) 0.06		

Of these figures we are inclined to attach the most value to those of experiments 17 and 18 for in these we are certain that there was the least degree of admixture of suprarenal blood with that from lumbar muscles. In experiment 17, the rate of flow was actually less during depressor stimulation than after: in experiment 18 the difference is far too small to account for the observed difference in epinephrine content.

We are reporting only nine of a series of eighteen experiments upon the subject under discussion: nine experiments have been discarded in the preparation of this report. Of these, five failed to show less epinephrine in the suprarenal blood taken during depressor stimulation than before or after. In two of these five the left splanchnic nerve was found to have been cut in the evisceration. In the four which contained positive results, the rate of blood flow from the suprarenal veins during depressor stimulation was increased to such an extent as to make it impossible to say that vascular change was not responsible for the diminution in epinephrine content. In not a single experiment has there been any evidence that the suprarenal blood taken during depressor stimulation contained more epinephrine than that taken before or after depressor stimulation.

From these results we are convinced that the processes in the suprarenal gland which are responsible for the discharge of epinephrine into the blood are subject to reflex inhibition by way of the depressor nerves: in a word, the mechanism of suprarenal secretion is involved not only in pressor but in depressor reflexes.

PROTOCOLS

In the following protocols the data for each blood collection are given in the following order: Number (designation) of blood sample; time of collection; volume of sample, its source, time which elapsed during collection, and rate of flow per second; blood pressure in millimeters of mercury at the beginning of collection.

Each tracing is to be read from left to right; contraction of the muscle is shown by the down stroke, relaxation by the upstroke of the lever. Each division of the time record represents 30 seconds and the actual time is printed at frequent intervals at the bottom of the tracing. Each tracing has been reduced to one-fourth of the size of the original. The numbers along the curves of muscle contraction are the numbers of blood samples tested: the exact time when each sample was substituted in the muscle chamber is shown by the point at which its number appears on the curve. Since the reproduction of the records has made some of the numbers almost illegible, those which designate suprarenal blood have been reprinted at the margin of the figure.

Experiment 1. October 13, 1914. Female rabbit, 2750 grams. Operations finished at 3.50 p.m. Artificial respiration. Blood samples as follows:

- B₁. 4.01. 2 cc. from cava, 22 seconds, 0.091 cc. per second. B. P., 80.
- B₂. 4.07. 3 cc. from suprarenal veins, 11 seconds, 0.272 cc. per second. B. P., 65.
- B₃. 4.12.30. 3 cc. from cava, 18 seconds, 0.166 cc. per second. B. P., 40.
- B₄. 4.21.33. 3 cc. from suprarenal veins during stimulation of left depressor (B. P., 40→25), 33 seconds, 0.091 cc. per second. B. P., 25.
- B₅. 4.27.30. 3 cc. from suprarenal veins, 18 seconds, 0.166 cc. per second. B. P., 30.
- B₆. 4.36.45. 3 cc. from suprarenal veins during stimulation of left depressor (B. P., 25→10), 23 seconds, 0.130 cc. per second.

In this experiment beginning with B₂, 3 cc. of Ringer's solution were injected into the jugular vein after each collection of blood.

The tracing from this experiment is shown in figure 1, page 62.

Experiment 2. October 15, 1914. Male rabbit, 2425 grams. Operation finished at 4.01 p.m. Artificial respiration. Blood samples taken as follows:

- 1. 4.15. 3 cc. from inferior cava; 43 seconds, 0.07 cc. per second. B. P., 40.
- 2. 4.27.52. 3 cc. from suprarenal veins during stimulation of left depressor nerve (B. P., 50→30), 33 seconds, 0.091 cc. per second. B. P., 30.
- 3. 4.32.40. 3 cc. from suprarenal veins, 42 seconds, 0.071 cc. per second. B. P., 35.

Between the collection of samples 2 and 3, 3 cc. of Ringer's solution were injected into the external jugular vein.

The tracing from this experiment is shown in figure 2, page 62.

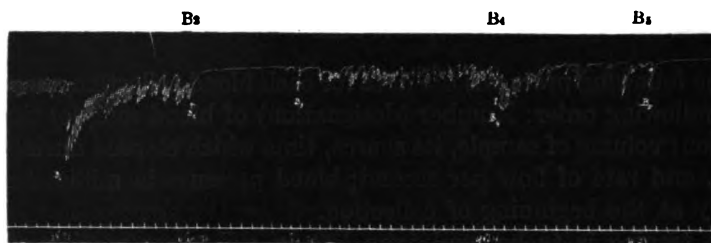


Fig. 1. *Experiment 1.* Cat's intestinal muscle contracting in Ringer's solution. At B₁, blood sample B₁ was substituted. The effects of samples B₂ and B₄ (suprarenal blood) are to be compared with that of B₄ (suprarenal blood taken during depressor stimulation).

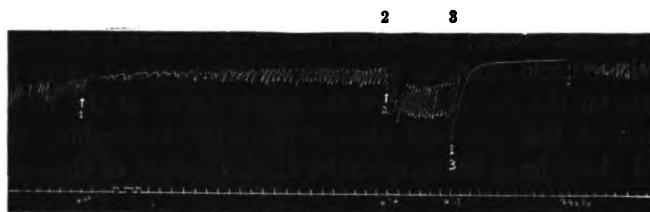


Fig. 2. *Experiment 2.* Cat's intestinal muscle contracting in Ringer's solution. Substitution of various blood samples is indicated by the figures below the tracing.

Experiment 3. October 16, 1914. Female rabbit, 2815 grams. Operations finished at 1.33 p.m. Artificial respiration. Blood samples taken as follows.

1. 1.26. 2 cc. from external jugular, 120 seconds, 0.016 cc. per second. B. P., 75.
2. 1.36.20. 3 cc. from suprarenal veins, 22 seconds, 0.136 cc. per second. B. P., 75.
3. 1.51.10. 3 cc. from suprarenal veins during stimulation of left depressor nerve (B. P. 60→40), 27 seconds, 0.111 cc. per second. B. P., 40.
4. 2.10.15. 3 cc. from suprarenal veins, 39 seconds, 0.077 cc. per second. B. P., 45.
5. 2.17.15. 3 cc. from suprarenal veins during stimulation of left depressor nerve (B. P., 42→18), 31 seconds, 0.097 cc. per second. B. P., 18.
6. 2.43. 3 cc. from inferior cava, 29 seconds, 0.104 cc. per second. B. P., 60.
7. 2.46.25. 2 cc. from suprarenal veins during a stimulation of left depressor nerve (B. P., 55→30), 26 seconds, 0.077 cc. per second. B. P., 30.
8. 2.52.35. 2 cc. from suprarenal veins, 34 seconds, 0.059 cc. per second. B. P., 40.
9. 3.00. 3 cc. from external jugular vein.

Between blood collections 5 and 6, 20 cc. of Ringer's solution were injected into the inferior vena cava.

The tracing from this experiment is shown in figure 3, page 63.



Fig. 3. *Experiment 3.* Cat's intestinal muscle contracting in blood sample 1. Substitutions of blood samples were made as indicated by figures under the tracings. The designation *RR* under the test of blood sample 2 means that the muscle was washed with Ringer's solution at the points indicated. At the beginning of the second portion of the tracing the muscle was contracting in blood sample 9.

Experiment 5. October 19, 1914. Female rabbit, 2825 grams. Operations finished at 3.12 p.m. Artificial respiration. Blood samples taken as follows:

1. 3.18. 3 cc. from external jugular, 80 seconds, 0.037 cc. per second. B. P., 48.
2. 3.27.30. 3 cc. from suprarenal veins during stimulation of left depressor nerve (B. P., 40→25), 33 seconds, 0.091 cc. per second. B. P., 25.
3. 3.42.15. 2.5 cc. from suprarenal veins, 33 seconds, 0.076 cc. per second. B. P., 35.
4. 4.23. 3 cc. from external jugular. B. P., 28.
5. 4.37. 3 cc. from suprarenal veins during stimulation of left depressor nerve (B. P., 40→18), 26 seconds, 0.115 cc. per second. B. P., 18.
6. 4.40.30. 3 cc. from suprarenal veins, 29 seconds, 0.103 cc. per second. B. P., 30.
7. 4.43. 2.5 cc. from external jugular. B. P., 25.

Between the collections of samples 3 and 4, 20 cc. of Ringer's solution were injected into the inferior vena cava.

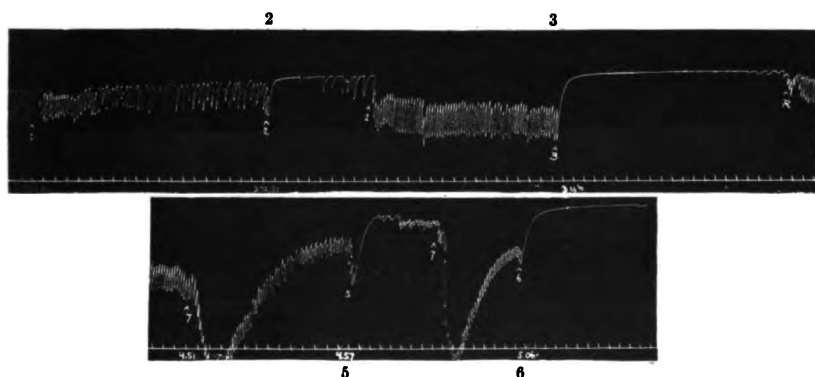


Fig. 4. *Experiment 5.* Cat's intestinal muscle contracting in Ringer's solution. Blood samples substituted as indicated by figures under the tracing. At the beginning of second portion of the tracing the muscle was contracting in blood sample 2 diluted with equal volume of Ringer's solution.

Experiment 11. November 19, 1914. Male rabbit, 1800 grams. Operations finished at 3.50 p.m. Natural respiration. Blood samples taken as follows:

1. 4.14. 3.2 cc. from external jugular, 7 minutes. B. P., 80.
2. 4.29. 1.8 cc. from suprarenal veins, 30 seconds, 0.06 cc. per second. B. P., 60.
3. 4.37. 1.8 cc. from suprarenal veins during stimulation of both depressor nerves (B. P., 40→28), 31 seconds, 0.058 cc. B. P., 28.
4. 4.50. 1.5 cc. from suprarenal veins, 51 seconds, 0.029 cc. per second. B. P., 22.



Fig. 5. *Experiment 11.* Cat's intestinal muscle contracting in Ringer's solution. Blood samples substituted as indicated by figures under the tracing.

Experiment 13. November 21, 1914. Male rabbit, 2100 grams. Operations finished at 11.43 a.m. Natural respiration. Blood samples taken as follows:

1. 11.57. 4.4 cc. from external jugular. B. P., 98.
2. 12.01. 3.4 cc. from suprarenal veins, 20 seconds, 0.170 cc. per second. B. P., 85.
3. 12.08.30. 1.8 cc. from suprarenal veins during central stimulation of left median nerve (B. P., 88→80), 22 seconds, 0.082 cc. per second. B. P., 80.
4. 12.13.30. 1.8 cc. from suprarenal veins during simultaneous stimulation of left median and of both depressor nerves (B. P., 70→65→47), 20 seconds, 0.09 cc. per second. B. P., 47.
5. 12.36. 2.2 cc. from suprarenal veins, 40 seconds, 0.055 cc. per second. B. P., 48.
6. 12.46. 1.6 cc. from suprarenal veins during stimulation of both depressor nerves (B. P., 55→45), 27 seconds, 0.059 cc. per second. B. P., 45.
7. 12.52. 1.8 cc. from suprarenal veins, 40 seconds, 0.045 cc. per second. B. P., 30.

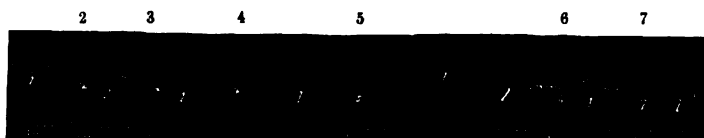


Fig. 6. *Experiment 13.* Cat's intestinal muscle contracting in Ringer's solution. Blood samples substituted as indicated by figures under the tracing. All blood samples were diluted with equal volume of Ringer's solution.

Experiment 14. November 24, 1914. Female rabbit, 2050 grams. Operations finished at 11.22 a.m. Natural respiration. Blood samples taken as follows:

1. 11.39. 4.6 cc. from external jugular. B. P., 85.

2. 11.52. 2.9 cc. from suprarenal veins, 26 seconds, 0.111 cc. per second. B. P., 80.
3. 12.01. 2.1 cc. from suprarenal veins during stimulation of left depressor nerve (B. P., 83→60), 25 seconds, 0.084 cc. per second. B. P., 60.
4. 12.11. 1.5 cc. from suprarenal veins during central stimulation of left median nerve (B. P., 75→70), 31 seconds, 0.05 cc. per second. B. P., 70.
5. 12.13. 1.8 cc. from suprarenal veins during simultaneous stimulation of left median and left depressor nerves (B. P., 80→70→50); 27 seconds, 0.066 cc. per second. B. P., 50.
6. 12.15. 1.8 cc. from suprarenal veins during central stimulation of left median nerve (B. P., 80→57→80), 30 seconds, 0.06 cc. per second. B. P., 80.

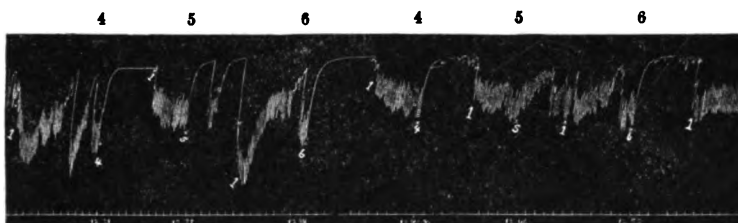


Fig. 7. *Experiment 14.* Cat's intestinal muscle contracting in blood sample 3. Other blood samples substituted as indicated by figures under the tracing. All blood samples were diluted with equal volume of Ringer's solution. During the interval between the first and second testing of samples 4, 5 and 6, they were kept in the air at room temperature under identical conditions.

Experiment 17. January 22, 1915. Female rabbit, 2200 grams. Operations finished at 11.50 a.m. Natural respiration. Blood samples taken as follows:

1. 12.07. 2.2 cc. from suprarenal veins immediately after the infusion of 25 cc. 0.9 per cent NaCl into vena cava (12.04-12.05.40), 6½ seconds, 0.35 cc. per second. B. P., 102.
2. 12.11. 2.1 cc. from suprarenal veins immediately after section of depressor nerves (B. P., 100→120), 9½ seconds, 0.225 cc. per second. B. P., 100.
3. 12.13. 3.5 cc. from external jugular vein. B. P., 110.
4. 12.38. 2 cc. from suprarenal veins, 25 seconds, 0.08 cc. per second. B. P., 70.
5. 12.44. 2.1 cc. from suprarenal veins during stimulation of both depressor nerves (B. P., 90→62), 30 seconds, 0.07 cc. per second. B. P., 62.

The tracing from this experiment is shown in figure 8, page 66.

Experiment 18. January 23, 1915. Rabbit, 1930 grams. Operations finished at 11.59 a.m. Blood samples taken as follows:

1. 12.35. 2 cc. from suprarenal veins immediately after infusion of 25 cc. of 0.9 per cent NaCl into inferior cava (12.30.30-12.32.10), 15 seconds, 0.133 cc. per second. B. P., 100.
2. 12.38. 2.2 cc. from suprarenal veins immediately after section of both depressor nerves (B. P., 100→118), 20 seconds, 0.110 cc. per second. B. P., 125.

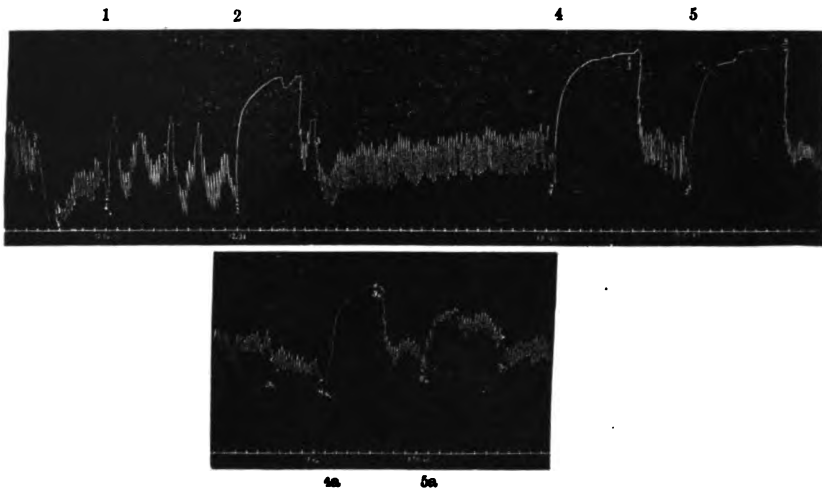


Fig. 8. *Experiment 17.* Cat's intestinal muscle contracting in Ringer's solution. Blood samples substituted as indicated by figures on tracing. In the tests shown in the first portion of tracing each blood sample was diluted with equal volume of Ringer's solution. In the second tests of bloods 4 and 5 (4a, 5a, second portion of tracing) each sample was diluted with 3 volumes of Ringer's solution. During the interval between the first and second tests of bloods 4 and 5 they were kept at room temperature under identical conditions.

3. 12.42. 1.8 cc. from suprarenal veins during stimulation of both depressor nerves (B. P., 120→120), 37½ seconds, 0.048 cc. per second. B. P., 120.

4. 12.43.30. 5.5 cc. from external jugular vein. B. P., 105.

5. 1.16. 2.2 cc. from suprarenal veins, 44 seconds, 0.05 cc. per second. B. P., 90.

6. 1.19. 2.3 cc. from suprarenal veins during stimulation of both depressor nerves (B. P., 85→70), 40 seconds, 0.057 cc. per second. B. P., 70.

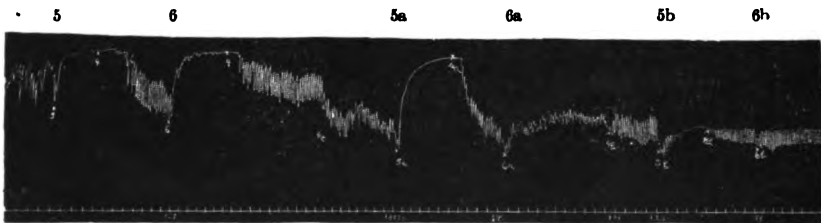


Fig. 9. *Experiment 18.* Cat's intestinal muscle contracting in blood sample 4 diluted with equal volume of Ringer's solution. Figures 5 and 6 indicate the substitution of samples 5 and 6, each diluted with one volume of Ringer's solution. Figures 4a, 5a, and 6a show tests of those samples diluted with 3 volumes of Ringer's solution. Figures 4b, 5b, and 6b indicate tests on the same samples, each diluted with 7 volumes of Ringer's solution.

A RESPIRATORY CHAMBER FOR SMALL ANIMALS

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The respiratory chambers which we wish to describe were specially devised for several lines of work which we desired to undertake in this laboratory. The central idea in all of this work depended on the development of an apparatus in which it would be possible to keep animals in a given atmosphere of oxygen and nitrogen continuously for a week or more. The chambers are suitable for small dogs, rabbits, cats, rats, mice, etc. It was necessary to have an apparatus which should be entirely automatic in regard to the oxygen supply and which would not require constant watching. It was not necessary for the work which we had in mind to determine the energy exchange. Although the apparatus is of particular interest in connection with our own work it seemed to be of sufficient general interest to warrant separate publication. We have built three chambers, two of galvanized iron and one of 3.18 mm. boiler plate steel. The larger one built of lighter material will be described first.

The larger chamber. This chamber is 90 cm. x 90 cm. x 45 cm. and is built of No. 22 B and S. gauge (0.635 mm.) (figs. 1 and 2), galvanized iron. There are two windows on opposite sides of the box, 45 cmf. x 30 cm. of double thickness glass held by strips of metal bolted through the sides of the box and made air tight by means of a 3 mm. rubber gasket, cement, and finally a coating of wax. There is an opening at the top of the box, 30 cm. x 30 cm. centrally placed. This opening is fitted with a door which is completely removable. The door is of heavy glass set in a brass frame and is held air-tight by means of six clamps which are easily opened so that the door can be taken off within a few seconds. A heavy rubber gasket is placed between the brass frame of this door and the brass casting which receives it. There is no trouble in getting this door air tight. The box is well lighted with the two large glass windows and glass door above. There is a small opening in the bottom through which urine or any fluid can be readily drained

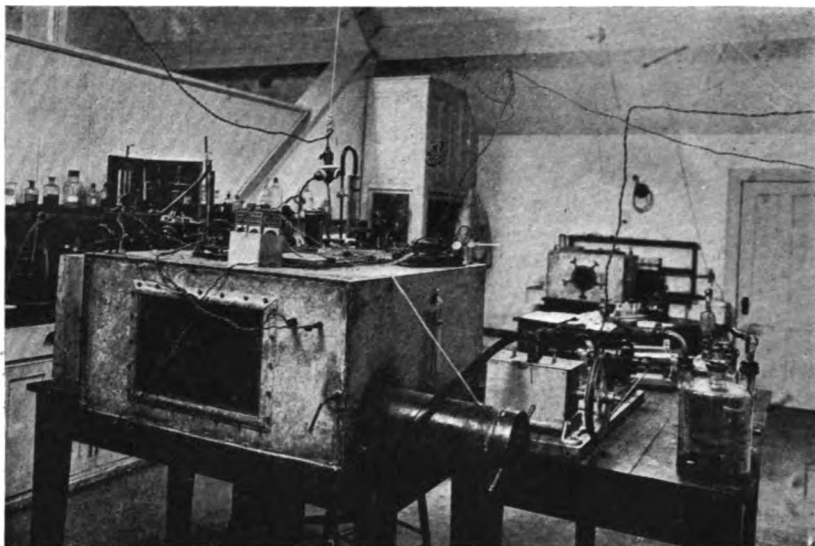
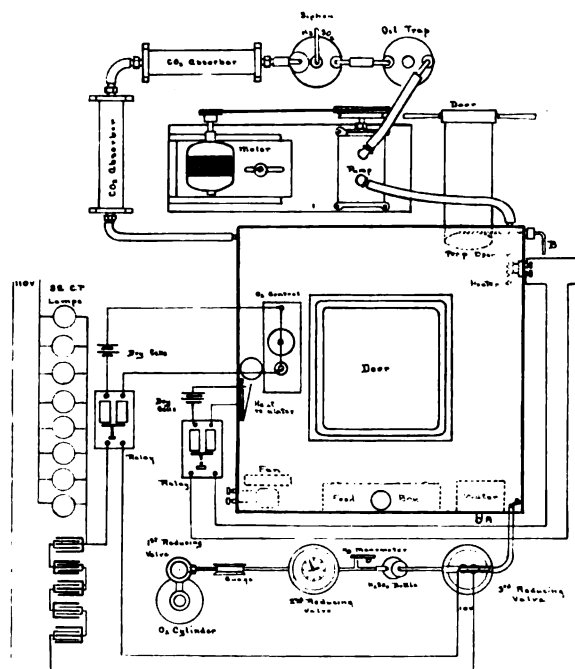


Fig. 1



Top View

Fig. 2

off, and if it were desirable obvious arrangements for flushing the box, from time to time, without opening it could be easily arranged. The arrangements for feeding depend on the animal used. There is a water sealed pipe leading through the side of the box to supply water (A, fig. 2). When rabbits are used we place in the box a hopper filled with corn, oats and ground alfalfa which feeds from the upper compartment into the lower. Enough feed may be placed in the hopper to last four rabbits for four days. To refill the hopper an opening was made in the top of the box, into which a supply pipe into the upper part of the hopper fits. This opening is closed by means of a large rubber stopper (5 cm. in diameter) and the food can readily be supplied for another four days by removing the stopper. This requires only a few seconds and the influence on the composition of the atmosphere of the box is entirely negligible.

Extending out from the box on the side on which the absorbing apparatus is placed is a trap made of iron piping 38 cm. long and 18 cm. in diameter. It is closed toward the outside by means of a door which screws on, a heavy rubber gasket being used between the bearing surfaces. This door is of glass with a heavy iron frame. At the entrance of the trap from the chamber is placed a door which may be opened and closed by means of a lever (B, fig. 2). The lever passes through a stuffing box to the outside. The trap serves a double purpose. When rabbits are used in the box they may be removed and examined without altering the composition of the atmosphere of the chamber by more than 1 per cent. This is done by closing the trap toward the interior of the chamber and removing the door. A carrot is placed just inside the trap and the door replaced. Then the trap is opened toward the interior of the chamber. Within a few minutes the rabbit goes into the trap attracted by the carrot, when the damper is closed and the rabbit is removed for observation. In case the rabbits are too timid to venture into the trap they can always be removed by a snare operated through the trap. Small dogs can also be readily removed through the trap. They come out willingly when given the opportunity. The other purpose of the trap is for the introduction of any special food material or drug from time to time. The apparatus for the absorption of the water and carbon dioxide produced by the animals is taken from Benedict¹ so that no description is required. We have, however, introduced three features of great convenience into the absorbing system, as follows:

¹ Benedict: Deutsch. Arch. f. klin. Med., 1912, cvii, 156.

First. Traps for the sulphuric acid bottle. (a) The tube entering the sulphuric acid bottle has a large bulb of 500 cc. capacity. In case the rubber tubing between the pump and sulphuric acid bottle should break this bulb would receive the sulphuric acid forced up by the back pressure in the rest of the absorbing system and prevent the concentrated acid from reaching the current of air from the pump and being sprayed over the room. (b) The outlet tube from the sulphuric acid bottle is provided with a trap having a large inner tube. The tube is lipped

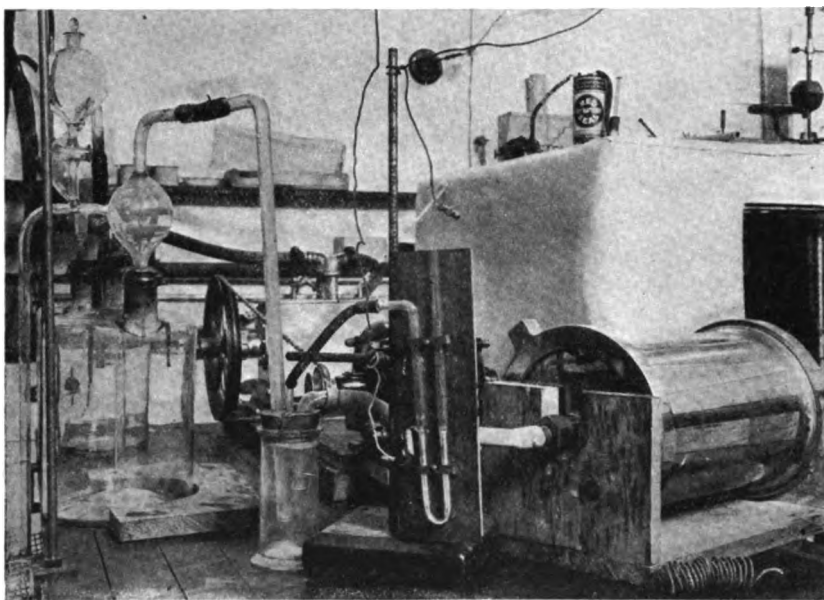


Fig. 3

at the bottom and allows the acid reaching the tube to flow back into the bottle without obstructing the current of air. This trap effectively prevents any acid from being carried over.

Second. In experiments of long duration it is a great convenience to be able to remove the spent sulphuric acid and to replace it with fresh acid without removing the stoppers. For this purpose the sulphuric acid bottle is provided with a combined siphoning tube and separatory funnel as shown in figure 3.

Third. A small bottle or T-tube is inserted between the sulphuric acid bottle and the soda-lime container (fig. 3). This bottle is con-

nected with a mercury manometer having a platinum wire fused into the glass and in contact with the mercury. Another platinum wire passes down the open end of the manometer. During the time that the apparatus is operating there is a positive pressure in the bottle—usually of about 10 mm. mercury. In case the soda-lime becomes caked and offers resistance to the passage of the air stream, it will be readily noted by the manometer reading. In case anything occurs to interfere with the circulation of the air, the manometer reading drops to zero and the mercury makes contact with the two platinum wires and closes a dry cell circuit which rings an electric bell. This obviates constant observation of the apparatus during the working hours. At night the wiring is slightly changed so that the first vibration of the electric bell releases a telephone connected with the university central (fig. 4). The signal from this 'phone is recognized by the university operator as a trouble signal and we are immediately notified. This obviates the necessity for having a man on duty constantly day and night during experiments.

The supply of oxygen.

This is automatically controlled as follows: A

10 cm. tambour covered with rubber dam of proper thickness is connected with the interior of the box. A decrease of the pressure within the box draws the rubber dam down. There is a brass arm bearing a platinum point resting upon a wedge glued to the center of the rubber dam. When the dam is drawn down, this arm closes a relay circuit which in turn breaks a circuit operating on the third oxygen valve described below. This is a slight modification of the method proposed by Williams.² Oxygen cylinders containing oxygen under a pressure of 100 to 150 atmospheres supply the oxygen as needed. The pressure is lowered by means of three reducing values. The first valve which is supplied with the cylinder reduces the pressure to approximately 6 atmospheres or less. We have inserted a gauge just beyond the

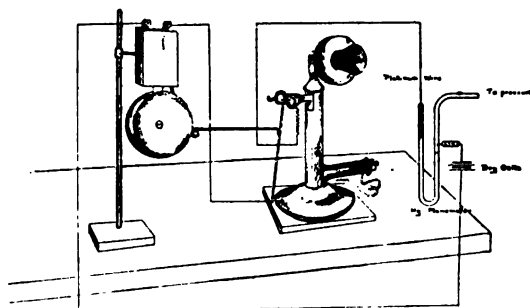
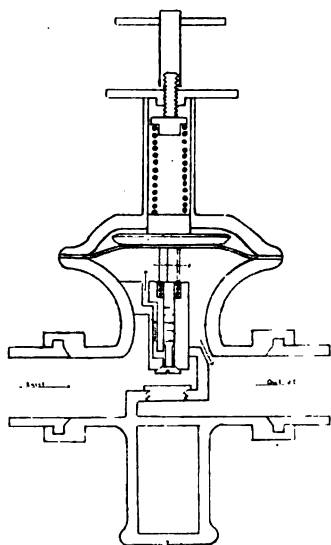


Fig. 4

² Williams: Journ. Biol. Chem., 1912, xii, 317.

valve so that the exact pressure at this point is known. The second and third reducing valves (figs. 5 and 6) were built by our mechanician from Mason reducing valves on the market. The first valve will operate between pressures of from 15 atmospheres to less than 1 atmosphere. We, however, never allow less than one atmosphere of pressure on the cylinder side of this valve. This valve is provided with a dial and pointer so that it may be set to deliver the oxygen towards the box at any desired pressure. In most of our work it was set to deliver oxygen at a pressure of approximately 30 mm. of mercury. A mercury



Second Oxygen Valve

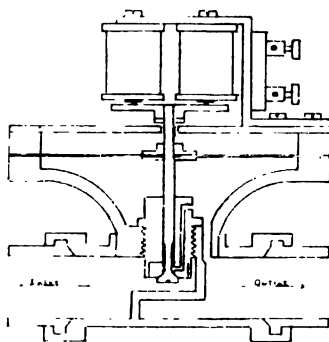
Fig. 5

manometer is placed between the second and third reducing valves in order to control perfectly the setting and working of this valve. The third valve opens or closes with changes of pressure within the box of 0.2 mm. of water. The electric control of this valve has already been described. When the pressure in the box falls due to the utilization of oxygen and the absorption of the water and carbon dioxide produced by the animal, contact is made between the tip of the tambour lever and the mercury cup. This actuates the relay which breaks the battery current. The valve then opens admitting oxygen into the box until the tip of the tambour lever leaves the mercury cup, when it is again closed. The box can be set for a pressure slightly above or slightly below atmospheric pressure by changing the position of the tambour lever on

the upright, or by raising or lowering the mercury cup. The box is provided with: A wet and a dry bulb thermometer and a simple arrangement for moistening the wet bulb; a 20 cm. electric fan playing on the thermometers in order to insure the maximum lowering of the wet bulb thermometer and to insure a uniform atmosphere in the box; an electric heater of nichrome wire controlled by a delicate heat regulating mechanism. This heater is not constructed to adjust for a very great fall in the temperature of the room and it is essential that the temperature of the room in which the chamber is placed should not vary more than 5°C. during an experiment. The temperature of the box is usually

about $1^{\circ}\text{C}.$ above that of the room during experiments. The importance of preserving a uniform temperature in the box will readily be understood when it is remembered that the system is entirely closed and the inflow of oxygen depends on changes in pressure within the system. Thus, if the temperature within the chamber falls, the per cent of oxygen will rise, whereas if the temperature rises the oxygen content will fall.

In the first work which we desired to do, the animal was to be placed in an atmosphere poor in oxygen at the ordinary atmospheric pressure and with as nearly as possible the normal humidity and carbon dioxide content of the outside air. The problem then presented itself as to the best method for quickly reducing the oxygen and raising the nitrogen content of the atmosphere of the box. We tried burning alcohol, phosphorus and illuminating gas, but all were unsatisfactory because of the production of pharmacologically active products which could not be completely absorbed. In the combustion of ethyl alcohol we found that aldehyde is produced among the products of oxidation as the oxygen concentration falls. Furthermore, alcohol and illuminating gas burn less and less vigorously as the oxygen content of the atmosphere decreases and they cease to burn at about 16 per cent and 13 per cent oxygen respectively.³ We also tried passing the air over highly heated copper turnings but found the method unsatisfactory. We found that by burning hydrogen in the atmosphere of the chambers we could reduce the oxygen content rapidly and in a manner entirely free of objections. During the time that the hydrogen is burned the chamber is left in communication with the outside air through a tube inserted at *M* (fig. 1), so that as the oxygen is absorbed by the flame and the water produced was removed by condensation and concentrated sulphuric acid, atmospheric air was constantly flowing into the chamber. We constructed a very convenient piece of apparatus for the burning



Third Oxygen Valve

Fig. 6

³ Illuminating gas varies so much in composition in different localities that it is not possible to state the oxygen percentage at which the flame is extinguished. The Madison illuminating gas is extinguished at about 13 per cent oxygen.

of the hydrogen and the condensation of the water (fig. 7) which is inserted in the absorbing system between the carbon dioxide absorber and the respiratory chamber. The chamber *A* in which the hydrogen is burned is 40 cm. high and 25 cm. in diameter. It is provided with a window, 4 cm. in diameter through which the flame could be observed. The volume of air delivered by the pump into the chamber varies from 32 to 52 liters a minute, depending on the speed of the pump. The current of air passes through the tube *B* and is directed toward the floor of the chamber. The air escapes from the chamber at the top and passes through the coil *C* and out at *D*. The sides of the chamber are continued upward 15 cm. so that the coil may be completely immersed in water and ice. The air then passes through the condensing chamber *E* which is packed in ice. It is shown in the dia-

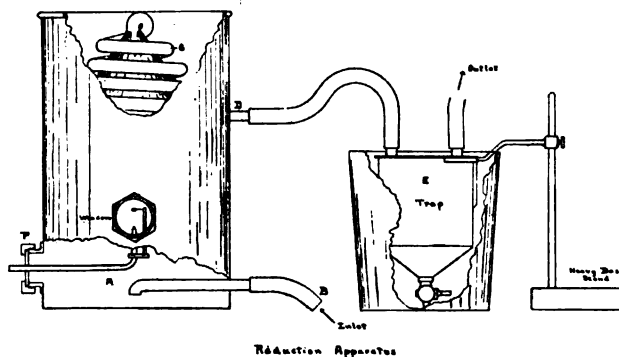


Fig. 7

gram in place in the pail. This apparatus is inserted without disconnecting any rubber tube. The hydrogen is burned from a brass tip and the flame impinges on a loop of platinum in order to make it luminous. This tip is held in place by a union, *F*. The hydrogen may be obtained from a Kipp apparatus or from a cylinder of hydrogen. Electrolytic hydrogen is now readily obtainable in most places. The advantages of hydrogen for reducing the oxygen are as follows: (1) The only product of the combustion is water. (2) If the flame is accidentally extinguished for a short interval the hydrogen does not interfere with the experiment since it is physiologically inert. (3) The hydrogen continues to burn until the oxygen in the atmosphere is reduced to about 6.6 per cent.

Using this apparatus we can reduce the oxygen in the large box to

10 per cent in thirty-five minutes and in the smaller box in twenty minutes.

A second method of reducing the oxygen in the chamber which we have used very extensively is to allow the animals to reduce the oxygen themselves. The rate of the reduction depends upon the rate of oxygen utilization by the animals. This method has the advantage that it enables the animals to become accustomed more gradually to the reduced partial pressure of oxygen but it occasions unnecessary loss of time.

A third method which would be free of objection would be to sweep the air out using compressed nitrogen, but unfortunately pure nitrogen is not always readily obtainable.

Chambers for work at pressures above and below atmospheric pressure

The smaller chamber which is 50 cm. x 50 cm. x 38 cm. is similar in its general appointments to the larger one except that it is made of heavier material, No. 18 galvanized iron (1 mm.). There are two round windows on opposite sides, 14 cm. in diameter. It is provided with an electric light of 2 c.p. which is only used during observations. This box is not provided with a trap for removing the animals. Both this chamber and the one previously described are covered with 2.5 cm. of wool pipe insulation material, then with heavy canvas and painted.

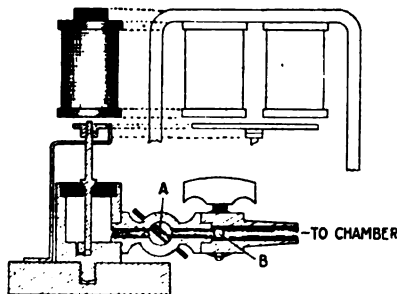


Fig. 8

The smaller chamber will stand a positive or negative pressure of about one-third of an atmosphere. The third chamber of 3.18 mm. boiler plate steel is oxy-acetyline welded along the seams. The chamber is round, the top being slightly concave and the bottom slightly convex. It is 76 cm. in diameter and 38 cm. in height. There are two round windows opposite one another and one in the center of the top, all 12 cm. in diameter. These windows are of 12 mm. plate glass, reinforced from the outside. This chamber was designed for work at reduced pressures and will stand complete evacuation. It will also stand a positive pressure of 10 kilograms per sq. cm. In the work at reduced pressure we have employed a Crowell rotary pump as in the experiments

at atmospheric pressure. Air is admitted to the chamber through the mechanism shown in figure 8. This mechanism has two openings, one controlled by a valve (*A*), and the other communicating continuously with the outside air (*B*), the size of both openings being regulated by stopcocks. Since the working principle of this mechanism depends on the gradual increase in negative pressure beyond the desired point, the stopcock controlling *B* is set so that this will occur. The other opening (*A*), which is controlled by the valve is alternately opened and closed by an electromagnet accordingly as the negative pressure increases and decreases. The current flowing through the coils of the electromagnet is automatically opened and closed by means of a relay. The relay circuit is in turn opened and closed through the contacts in the mercury manometer, which consist of platinum wire, one sealed in the side and the other introduced in the open end. The entrance of air and the pressure within the chamber is thereby automatically controlled and requires no attention whatever. The same alarm system described in connection with the first chamber is also in use with this chamber.

The results of our work with the chambers will be presented in subsequent communications.

THE MECHANISM ADAPTING THE OXYGEN CAPACITY OF THE BLOOD TO THE REQUIREMENTS OF THE TISSUES

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In 1878 Paul Bert¹ made the remarkable prediction that the blood of man and animals living at high altitudes would be found to have a greater oxygen capacity than that of corresponding individuals living at lower levels. He surmised that the cause of this increase in the oxygen carrying power of the blood would be found to be the decrease in the partial pressure of oxygen in the atmosphere respired, and that this constitutes the important feature of acclimatization to high altitudes. He believed that this adaptation would occur only after several generations of residence at high altitude. In 1882 Bert² confirmed his prediction and made the fundamental observation that the oxygen carrying power of the blood is much enhanced in animals living at high altitudes. The increase in the oxygen capacity of the blood could only be attributed to an increase in its haemoglobin content.

Viault³ in 1890 showed in man that on passing from a lower to a higher altitude there occurs within a short time a marked increase in the erythrocytes in the unit volume of blood. This completed Bert's fundamental discovery. The observations of Bert and Viault have been controverted by a few investigators but at the present time the evidence that the increases in erythrocytes and haemoglobin do occur is overwhelming. The physiological significance and the mechanism of these blood changes have been the subject of a great number of investigations

¹ Bert: "La pression barometrique," Paris, 1878, p. 1108.

² Bert: Compt. rend. de l'Acad. d. Sci., 1882, xciv, 805. Bert states that Jourdanet was of the opinion that mountain sickness is due to diminished oxygen in the blood consequent to the decreased oxygen tension of the atmosphere and he designated that state of the organism as "anoxyhémie."

³ Viault: Compt. rend. de l'Acad. d. Sci., 1890, cxi, 917; 1892, xciv, 1562. Compt. rend. de la Soc. de Biol., 1892, ix, Sér. iv, 569.

and the source of much controversy. It was with the view of throwing light on these propositions that the present study was undertaken. It was our desire to determine, by methods which would be free from objection, the nature of the blood changes at decreased pressure and if possible to bring this remarkable adaptation in line with other better known if not better understood physiological phenomena.

Historical. It is not our intention to present or critically review all of the researches in this field of work. Several excellent summaries of this literature have been published. We shall only refer to such contributions as are necessary in order to present our own results in the proper perspective.

There may be said to be unanimity among the recent workers that marked increases in erythrocytes and haemoglobin occur at high altitudes. Views differ as to the physiological significance of these changes and the mechanism by which they are brought about. Cohnheim⁴ is the only well-known recent worker who has expressly maintained that the blood changes are of but little physiological significance and he has completely reversed his decision in his last communication.⁵ The views in regard to the mechanism by which the blood changes are brought about may be conveniently divided into three main classes:

I. Those theories which insist that the increase in erythrocytes and haemoglobin is real and not merely relative. Two explanations of the increase have been proposed: (a) That the increase is due to increased activity of the bone marrow resulting in an increase in the erythrocytes and haemoglobin. This was the opinion held by Bert,⁶ accepted by Vaiult⁷ and elaborated by Miescher⁸ who held that the stimulation of the bone marrow must be attributed to the low oxygen tension in the bone marrow at high altitudes. (b) That the increase is due to a lengthening of the life of the erythrocyte as advanced by Fick⁹ has never received any experimental support and since everything points the other way this view may be disregarded.

II. The concentration theories according to which the increase in erythrocytes and haemoglobin per unit volume is due to increased concentration of the blood. According to this view the increase in erythro-

⁴ Cohnheim: *Ergebn. d. Physiol.*, 1912, xii, 629.

⁵ Cohnheim and Weber: *Deutsch. Arch. f. klin. Med.*, 1913, cx, 225.

⁶ Loc. cit.

⁷ Loc. cit.

⁸ Miescher: *Korrespondenzblt. f. Schweizer Aerzte*, 1893, xxiii, 809.

⁹ Fick: *Arch. f. d. gesamt. Physiol.*, 1895, lx, 589.

cytes and haemoglobin is only apparent and there is no increase in the total erythrocytes and haemoglobin in the body. This view was first advanced by Grawitz¹⁰ who maintained that there occurs increased evaporation of water from the body at high altitudes and that this results in increased concentration of the blood. If this view were correct, it would be found:

1. That the increases in erythrocytes and haemoglobin would always run parallel.

2. That since the increases in erythrocytes and haemoglobin often amount to 20 per cent, the blood would have to lose $16\frac{1}{3}$ per cent of its water and since the blood under these conditions would take up water from the tissues, in consequence of its increased tonicity, the entire body would have to lose $16\frac{1}{3}$ per cent of its water which in a man of 70 k. would mean a loss of weight of 8.2 k.

3. When the body had lost the amount of water calculated above (8.2 k) there would necessarily be a marked increase in the specific gravity of the plasma and in the material in solution.

4. A determination of the total haemoglobin in the body would show that there is no increase in the total haemoglobin.

None of these propositions are in accord with the facts. Abderhalden¹¹ alone found a marked parallelism in the changes in erythrocytes and haemoglobin. Some workers have found the erythrocytes increased more than the haemoglobin, while others have found the haemoglobin to show the greater increase. Another striking argument against the view that the increases are not due to loss of water by evaporation, if any further evidence were required, is to be found in the regulation of the degree of hydraemia by the kidneys which ordinarily take care of a very large variation in the water ingestion with practically no changes in the blood count. Increased evaporation without an increase in ingestion of water would result in the secretion of very concentrated urine.

The theory of Grawitz was modified by Bunge and others to meet some of the above objections. Bunge¹² supposed that vaso-constriction occurs at high altitude resulting in plasma leaving the vessels and passing into the tissues. This met the objection that there is no increase in the solid contents of the plasma at high altitudes. However, there is no evidence of such long continued vaso-constriction

¹⁰ Grawitz: Berl. Klin. Wochens., 1895, xxxii, 713, 740.

¹¹ Abderhalden: Zeit. f. Biol., 1902, xliii, 125.

¹² Bunge: Verhandl. des XIII Kongress f. innere Med., 1895, p. 192.

and the only experimental evidence favoring this view is the parallelism between the increases in erythrocytes and haemoglobin noted by Abderhalden and which has not been confirmed by other workers. The fallacy of both of these views is most clearly brought out by determinations of the total haemoglobin in experimental animals which was done by Jaquet and Suter¹³ and Abderhalden.¹⁴ Abderhalden attached no significance to the increase in haemoglobin per kilo which he found in animals at high altitude. It should be stated that Abderhalden in his more recent communication¹⁵ on this subject states guardedly that the increases in the blood count may not be fully accounted for on the theory of increased concentration of the blood and states that new formation of erythrocytes occurs without doubt especially when there is prolonged residence at high altitude. Douglas, Haldane, Henderson and Schneider¹⁶ in an exhaustive study of the blood changes on Pike's Peak conclude that the increased percentage of haemoglobin was apparently due in part during the first few days to concentration of the blood but afterwards entirely to a large increase in the total amount of haemoglobin which was determined by the carbon monoxide method. Schneider and Havens,¹⁷ also working on Pike's Peak, find that the increase in erythrocytes and haemoglobin during the first two or three days at high altitude is due in part to the throwing into the general circulation of a large mass of reserve corpuscles, in part to a loss of fluid from the blood and to increased activity of the bone marrow.

III. Under this class may be grouped every other conceivable explanation of the observed facts. Thus it has been stated that the increase in the erythrocyte count at high altitude is due to a distortion of the cover slip so that the depth of the counting chamber is increased. This view is founded on faulty theoretical reasoning and has been shown experimentally to be erroneous. Again it has been held that the increases are due to unequal distribution of erythrocytes. They were supposed to be more numerous in the blood of the capillaries and smaller vessels and less numerous in the large vessels. This view has found but insignificant support experimentally and the mass of obser-

¹³ Jaquet and Suter: *Correspondenzblt. f. Schweizer Aerzte*, 1898, xxviii, 104.

¹⁴ Abderhalden: *Zeit. f. Biol.*, 1902, xliii, 125.

¹⁵ Abderhalden: *Med. Klinik*, 1905, 1, 210.

¹⁶ Douglas, Haldane, Henderson and Schneider: *Phil. Trans. Royal Soc.*, 1913, B. cciii, 185.

¹⁷ Schneider and Havens: *This Journal*, 1915, xxxvi, 380.

vations opposed to it render it untenable. Finally, it has been supposed that there exists in the body a reserve or dormant supply of erythrocytes which is drawn upon at high altitude.

Methods. Previous work carried on in this laboratory has demonstrated that within certain limits the activity of the respiratory, vaso-motor and cardio-inhibitory centers may be stimulated by reducing oxygen fixation by the cells of these centers.¹⁸ To express ourselves more accurately, the results obtained could be satisfactorily explained only on the basis of the above hypothesis. Various considerations have led us to the view that the above conclusion holds good generally for living cells and this leads directly to the proposition that the bone marrow should be stimulated by any means which will within certain limits decrease the oxygen fixation of its cells. It seemed therefore that the views of Bert and Miescher were entirely in accord with the work on the medullary centers and we determined to put them to the test.

There are many conceivable methods of reducing oxygen fixation by the bone marrow. Substances such as hydrocyanic acid which are known to reduce oxidation in living cells might be used in which case the supply of oxygen need not be reduced. There are also many methods which might be employed for reducing the oxygen supplied to the bone marrow. Nasmith and Graham¹⁹ and Nasmith and Harrison²⁰ have studied the effect of chronic carbon monoxide poisoning on guinea pigs and rabbits and found marked increases in the erythrocytes and haemoglobin. Nasmith and Graham using guinea pigs state, "the effect of chronic carbon monoxide poisoning in the blood is similar to that which occurs at high altitude." Their studies were apparently not made with a view to determine the cause of the polycythaemia of altitude and are therefore the more convincing that decreased oxygen supply to the bone marrow leads to increased production of erythrocytes and haemoglobin.

Bürker, Ederle and Kircher²¹ devised a different method of decreasing the oxygen supply to the bone marrow. They lessened the respiratory surface of the lungs by means of a unilateral pneumothorax. Their

¹⁸ Grove and Loevenhart: *Journ. Pharm. and Exp. Therap.*, 1911, iii, 131. Loevenhart: *Arch. f. d. gesamt. Physiol.*, 1913, cl, 379. Gasser and Loevenhart, *Journ. Pharm. and Exp. Ther.*, 1914, v, 239.

¹⁹ Nasmith and Graham: *Journ. Physiol.*, 1906, xxxv, 32.

²⁰ Nasmith and Harrison: *Journ. Exp. Med.*, 1910, xii, 282.

²¹ Bürker, Ederle and Kircher: *Zentralblt. f. Physiol.*, 1913, xxvii, 623.

observations were made on men, dogs, and rabbits, and they found marked increases in the erythrocytes and haemoglobin. The method is open to the objection that with the collapse of one lung a marked abnormality is introduced.

In our experiments we have reduced the oxygen supply to the bone marrow by lessening the oxygen tension of the respired air. This was accomplished by two different methods, which served to check one another. First we have exposed animals to atmospheres varying in composition from 6 per cent by volume of oxygen and 94 per cent of nitrogen to 21 per cent oxygen and 79 per cent nitrogen at atmospheric pressure and second, we have worked at the corresponding tensions of oxygen obtained by partially evacuating the chamber in which the animals were kept. The apparatus in which the work was carried out is described in the preceding article together with the automatic devices which enabled us to carry out the experiments readily. In some cases we have kept the animals in the chambers described for a month only removing them every five or seven days for a few hours in order to keep the chambers in a thoroughly hygienic condition. In most of our work the animals were kept in the chambers from two to seven days. In the experiments at atmospheric pressure with reduced oxygen tension there were slight variations in the composition of the atmosphere. They were sufficiently small, however, to be disregarded entirely. They were practically always due to changes in temperature. From two to four analyses daily for oxygen and carbon dioxide enabled us to correct changes in the atmosphere before they assumed any proportions. The carbon dioxide analyses were made with Haldane's intermediate size portable apparatus for the analysis of mine gases. The oxygen determinations were made in the usual way with a Hempel phosphorus pipette as large volumes of gas could be readily taken. The importance of the experiments at atmospheric pressure using atmospheres of low oxygen tension are sufficiently obvious. Our object was to remove any mechanical factors, such as exist at reduced atmospheric pressure and which have been frequently evoked to account for the blood changes at high altitude. Thus increased evaporation of water from the body at reduced pressure has been regarded as an important factor in the high blood counts of altitude. Kronecker²² has assumed that at high altitude the diaphragm occupies a higher

²² Kronecker: "Die Bergkrankheit." v. Leyden's u. Klemperer's Die deutsche Klinik am Eingange des 20 Jahrhunderts 1907, 11, 130.

position and lessens the circulation through the lungs. Jacobi stated²³ that congestion of the lungs at high altitude causes a new formation of blood to fill the remainder of the vascular system. Durig and N. Zuntz²⁴ found that on the peak of Teneriffa their vital capacity was decreased 6 per cent to 11 per cent. Meyer²⁵ and Strohl²⁶ using the electrocardiographic method found an hypertrophy of the right ventricle at high altitudes. Heger²⁷ holds a view quite similar to that of Kronecker.

All theories which account for the blood changes by the reduced atmospheric pressure apart from decreased oxygen tension could be dismissed if it could be shown that the same changes occur at atmospheric pressure provided the oxygen tension is reduced by increasing the percentage of the inert gases of the atmosphere. This has been attempted by two previous investigators. Twenty years ago Sellier²⁸ attempted a similar solution of the problem. He used birds and guinea pigs. The experiments were apparently all of short duration. He states that he was forced to publish unwillingly before the work was completed. His apparatus was so extremely crude judging from the very meagre description that very little importance can be attached to his experimental findings. His conclusion that the polycythaemia of high altitude is due solely to low oxygen tension and that the reduced pressure, *per se*, exercises no influence, is interesting in spite of the fact that the data were not sufficient to establish the point. Recently David²⁹ has made similar studies. His apparatus is more elaborate but some of the methods used by him do not seem to be all that could be desired for experiments of long duration. Thus he uses potassium hydroxide for the absorption of carbon dioxide and calcium chloride for the absorption of water, both apparently in the same container. He performed a large number of experiments on animals previously rendered anaemic. The number of experiments on normal animals which he published is not sufficient to determine the reaction of normal bone marrow to oxygen want.

²³ Jacobi: Deutsch. Med. Woch., 1907, xxxiii, 17; Arch. f. exp. Path. u. Pharm., 1914, lxxvi, 423.

²⁴ Durig and N. Zuntz: Biochem. Zeitsch., 1912, xxxix, 435.

²⁵ Meyer: Journ. Med. de Bruxelles, 1912, 17, 409, 424.

²⁶ Strohl: Atti. d. lab. scien., A. Mosso Torino; 1912, iii, 218.

²⁷ Heger: Journ. Med. de Bruxelles, 1912, No. 46.

²⁸ Sellier: Thèse, Doc. Med. Bordeaux, 1894-5.

²⁹ David: Zeitsch. f. klin. Med., 1912, lxxiv, 404; Deutsch; Archiv. f. klin. Med., 1913, cix, 129.

The methods of operating our apparatus are fully described in the previous paper and also the method used for obtaining the desired atmosphere for the particular experiment in our apparatus. We have performed seventy-six experiments on sixty-one animals. The animals used were as follows: twenty-three full grown, eighteen half-grown and six young rabbits, two young pups and twelve half-grown, white rats. By far the greater number of our experiments were performed on rabbits. The samples of blood were usually taken from the marginal ear vein in rabbits and dogs but some counts were also made in rabbits from the carotid blood, or the blood in the heart, in order to determine whether there was any difference between the blood counts in the peripheral blood and that of the larger vessels. In the case of the rats the blood was obtained from the heart. The erythrocyte counts were made with the same pipettes throughout the investigation. Thoma-Zeiss counting chambers were used exclusively and here again we have used the same chambers throughout the work. Thus, since we were concerned with changes in the blood count and not absolute values, errors due to apparatus are excluded. The diluting solution used was principally a 5 per cent solution of magnesium sulphate. In the case of very young animals it was found necessary to slightly modify this solution in order to prevent the destruction of erythrocytes. All haemoglobin determinations were made with the same v. Fleischl-Miescher haemoglobinometer, the same Miescher pipette being employed throughout. All erythrocyte counts and haemoglobin determinations were made at least by two and often by all three of us, and the average of the results was taken. In case of any marked disagreements new determinations were made. In four of the rats, two at low oxygen tension and two controls at normal oxygen tension, determinations of the total haemoglobin were made. The animals were anaesthetized with ether and were perfused with Ringer's solution through the aorta until the perfusate was no longer blood stained. The tissues were then cut up and extracted with the same solution. The haemoglobin was then determined in the total mixture using the Miescher method. Everytime blood was taken smears were prepared and studied later. Jenner stain was employed, because of its characteristic staining of newly formed erythrocytes. Many of the rabbits were killed at the end of the experiments and the bone marrow and various other tissues preserved in formaldehyde or Zenker's fluid for histological study.

EXPERIMENTAL PART

Experiments at atmospheric pressure. These experiments constitute the major portion of our work. Here all the accessory effects of altitude such as excessive light, temperature changes, increased evaporation, reduced carbon dioxide tension and reduced barometric pressure are eliminated and we have but one factor in common with high altitude, namely, reduced partial pressure of oxygen. The methods of obtaining the particular atmosphere desired for study is described in the previous paper. For purposes of illustration we here give the protocol of one complete and typical experiment; the results in the remaining experiments will be merely summarized.

In all of the tables which follow we have expressed the haemoglobin in grams per 100 cc. of blood. In order to translate this figure into the ordinary clinical scale (100 per cent = normal) the figures given should be multiplied by 7.14.

EFFECT OF AN ATMOSPHERE OF 10 PER CENT OXYGEN AT
ATMOSPHERIC PRESSURE

March 21, 1914. Rabbit No. 7. Weight, 1620 g.

Haemoglobin (dilution 1 : 200). Scale: 66.5—12.4 gms. per 100 cc. blood.

Erythrocyte counts:

7,176,000	} Average 6,979,000
6,936,000	
6,824,000	

Rabbit No. 8. Weight, 1650 g.

Haemoglobin (dilution 1 : 200). Scale 91.1—16.9 gms. per 100 cc. blood.²⁰

Erythrocyte counts:

6,304,000	} Average 6,438,000
6,280,000	
6,686,000	
6,480,000	

March 22, 1914.

11.50 a.m. Rabbits placed in chamber. The circulation of air through soda lime and sulphuric acid started. The chamber communicated with the outside air. No oxygen admitted. The animals gradually reduced the oxygen content of the atmosphere in the chamber.

11.30 p.m. Oxygen 13.6 per cent.

²⁰ These animals had been previously subjected to another experiment. Rabbit 8 had not returned to normal condition which accounts for the very high concentration of haemoglobin noted.

March 23, 1914.

7.10 a.m. Oxygen turned on. Chamber closed to outside air.
8.30 a.m. Oxygen 9.7 per cent.
10.00 a.m. CO₂, 0.15 per cent.
12.15 p.m. O₂, 10.7 per cent.
8.45 p.m. O₂, 13 per cent.
8.50 p.m. CO₂, 0.15 per cent.
9.30 p.m. Humidity, 23 per cent.

March 24, 1914.

9.30 a.m. O₂, 13.9 per cent.
9.55 a.m. O₂ turned off.
10.00 a.m. CO₂, 0.17 per cent.
11.30 a.m. CO₂, 0.18 per cent. CO₂ absorber changed.
1.20 p.m. O₂, 11.1 per cent; O₂ turned on.
2.30 p.m.-4.30 p.m., O₂ turned off.
8.30 p.m. O₂, 10.2 per cent.

March 25, 1914.

10.20 a.m. O₂, 10.4 per cent.
10.30 a.m. CO₂, 0.07 per cent.
10.50 a.m. CO₂, 0.05 per cent.
11.30 a.m. Humidity, 32.5 per cent.
1.00 p.m. CO₂, 0.05 per cent.
7.40 p.m. O₂, 10.3 per cent.

March 26, 1914.

9.50 a.m. O₂, 10.6 per cent.
10.00 a.m. Humidity, 37.0 per cent.
11.30 a.m. CO₂, 0.13 per cent.
8.40 p.m. O₂, 11.7 per cent.
9.20 p.m. CO₂, 0.1 per cent.

March 27, 1914.

9.40 a.m. O₂, 14.4 per cent.
9.50 a.m. CO₂, 0.23 per cent.
10.00 a.m. O₂ turned off.
11.30 a.m. CO₂, 0.19 per cent.
2.30 p.m. Changed CO₂ absorber.
3.15 p.m. O₂, 12.1 per cent.
7.00 p.m. O₂, 10.6 per cent.
7.10 p.m. CO₂, 0.09 per cent.
7.45 p.m. O₂ turned on.

March 28, 1914.

11.30 a.m. O₂, 10 per cent.
2.15 p.m. CO₂, 0.08 per cent.
2.45 p.m. Humidity, 42 per cent.
7.10 p.m. O₂, 10.2 per cent.
7.25 p.m. CO₂, 0.1 per cent.

March 29, 1914.

10.30 a.m. O₂, 10.3 per cent.
10.45 a.m. CO₂, 0.11 per cent.

11.00 a.m. Humidity, 39 per cent.

6.55 p.m. O₂, 11.3 per cent.

7.10 p.m. CO₂, 0.14 per cent.

March 30, 1914.

2.00 p.m. O₂, 12.9 per cent.

2.00 p.m.-8.00 p.m. O₂ turned off.

4.30 p.m. CO₂, 0.11 per cent.

8.10 p.m. O₂, 10.4 per cent.

March 31, 1914.

1.30 p.m. O₂, 11.2 per cent.

1.40 p.m. CO₂, 0.15 per cent.

2.00 p.m. Rabbits removed from box.

Rabbit No. 7. Weight, 1550 g. Loss of weight, 70 g.

Haemoglobin (dilution 1 : 200). Scale: 92.3 - 17.2 g. per 100 cc. blood. Increase of 4.8 g. = 38.6 per cent.

Erythrocyte counts:

$$\left. \begin{array}{l} 7,752,000 \\ 7,824,000 \\ 7,328,000 \end{array} \right\} \text{Average } 7,635,000. \text{ Increase of } 656,000 = 9.4 \text{ per cent.}$$

Rabbit No. 8. Weight, 1620 g. Loss of weight 30 g.

Haemoglobin (dilution 1 : 200). Scale: 104.7 - 19.5 g. per 100 cc. blood. Increase of 2.6 g. = 15 per cent.

Erythrocyte counts:

$$\left. \begin{array}{l} 8,696,000 \\ 8,560,000 \\ 8,256,000 \end{array} \right\} \text{Average } 8,504,000. \text{ Increase of } 2,066,000 = 32 \text{ per cent.}$$

TABLE 1

*Control experiments at atmospheric pressure with normal oxygen pressure
(80.8 per cent)*

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			per cent			per cent
Rabbit No. 7...	1580	1560	- 20	6.7	6.8	+1.5	11.6	13.9	+19.8
8...	1300	1500	+200	7.1	7.1	0	14.2	16.6	+16.9
9...	852	970	+118	5.3	5.8	+9.4	10.9	11.6	+ 6.4
10...	861	935	+ 74	6.4	6.5	+1.6	13.1	13.7	+ 4.6
11...	790	898	+108	6.2	5.8	-6.4	12.7	11.5	- 9.4
Dog No. 1...	2140	2080	- 60	5.7	5.9	+3.5	10.3	11.2	+ 8.7
2...	3520	3320	-200	5.4	5.9	+9.3	10.2	12.3	+20.6
Average percentage blood change.....						+2.7			+9.7

The recorded variations in the oxygen were due to marked changes in the temperature of the room which the heat regulating mechanism could not take care of. However, in an experiment of this duration it is very difficult indeed to prevent some variations and we have presented the experiment in detail as a fair average of what we have been able to accomplish with the apparatus without extraordinary care. We have in many experiments kept the oxygen content within very much narrower limits.

TABLE 2

Atmospheric pressure, oxygen 16 per cent

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			per cent			per cent
Rabbit No. 29...	1740	1480	-260	4.7	4.5	-4.3	11.0	8.4	-23.6
30...	1420	1610	+190	4.5	4.9	+8.8	9.2	10.1	+9.8
31...	1510	1690	+170	5.7	6.4	+12.3	11.8	11.7	-0.8
32...	1400	1500	+100	6.8	6.9	+1.5	12.7	13.6	+7.1
Average percentage blood change						+4.6			-2.0

TABLE 3

Atmospheric pressure, oxygen 14 per cent

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			per cent			per cent
Rabbit No. 30...	1610	1540	-70	4.9	4.8	-2.0	10.1	10.8	+6.9
31...	1680	1720	+40	6.4	6.6	+3.1	11.7	14.6	+24.8
32...	1500	1510	+10	6.9	6.9	0	13.6	14.0	+2.9
39...	1500	1450	-50	5.0	7.1	+42.0	12.4	14.1	+13.7
40...	1550	1780	+230	5.2	7.2	+38.5	11.9	15.6	+31.1
41...	1600	1720	+120	5.7	7.2	+26.3	11.8	15.6	+32.2
42...	1440	1380	-60	5.3	5.5	+3.8	12.0	13.0	+8.3
43...	1620	1560	-60	5.2	5.7	+9.6	10.7	13.0	+21.5
Average percentage blood change						+15.2			+17.4

TABLE 4

Atmospheric pressure, oxygen 12 per cent

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			<i>grams</i>			<i>per cent</i>			<i>per cent</i>
Rabbit No. 5...	1760	1700	— 60	6.7	8.4	+25.4	14.0	17.0	+21.4
6...	1560	1600	+ 40	8.4	8.5	+ 1.2	14.4	17.4	+20.8
30...	1540	1670	+130	4.8	5.5	+14.6	10.8	12.3	+13.9
31...	1720	1840	+120	6.6	7.1	+ 7.6	14.6	16.3	+11.6
32...	1510	1470	— 40	6.9	8.5	+23.2	14.0	16.3	+16.4
Average percentage blood change						+14.4			+16.8

TABLE 5

Atmospheric pressure, oxygen 10 per cent

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			<i>grams</i>			<i>per cent</i>			<i>per cent</i>
Rabbit No. 7...	1620	1550	— 70	7.0	7.6	+ 8.6	12.4	17.2	+38.7
8...	1650	1620	— 30	6.4	8.5	+32.8	16.9	19.5	+15.4
9...	970	1060	+ 90	5.8	7.5	+29.3	11.6	15.7	+35.3
10...	935	1070	+135	6.5	7.3	+12.3	13.7	16.4	+19.7
11...	898	973	+ 75	5.8	7.3	+25.9	11.5	17.0	+47.8
12...	1200	1220	+ 20	6.9	7.8	+13.0	14.6	16.6	+13.7
13...	1280	1300	+ 20	6.8	7.1	+ 4.4	12.0	16.2	+35.0
14...	1120	1210	+ 90	6.9	7.2	+ 4.3	13.3	15.6	+17.3
15...	1050	1100	+ 50	5.4	6.6	+22.2	10.6	16.8	+58.5
16...	1010	1095	+ 85	6.0	7.0	+16.7	11.2	15.0	+33.9
22...	1400	1140	—260	6.7	7.1	+ 6.0	14.7	15.9	+ 8.2
23...	1320	1200	—120	5.7	7.6	+33.3	13.8	15.1	+ 9.4
24...	1420	1350	— 70	6.5	7.8	+20.0	13.9	15.6	+12.2
Dog* No. 1...	2420	2460	+ 40	6.6	7.6	+15.2	11.8	13.7	+16.2
2...	3820	3520	—300	6.1	8.2	+34.4	10.2	14.3	+40.2
Average percentage blood change						+18.5			+26.7

* The experiments with dogs 1 and 2 lasted 12 days.

Experiments at low barometric pressure. In these experiments we have eliminated all the factors characteristic of high altitude except reduced atmospheric pressure and the corresponding reduction of the partial pressure of oxygen which this entailed. These experiments were performed in order to determine whether reduced barometric pressure plays any rôle in the polycythaemia of altitude apart from the reduced partial pressure of oxygen. If the effects of reduced barometric pressure are dependent solely on the reduced partial pressure of oxygen then the results should be identical at atmospheric pressure and at reduced pressure when the same partial pressure of oxygen prevails. We shall not present a protocol of one of these experiments because there never occurred any deviation in the pressure or in the composition of the atmosphere of the chamber from that which we desired to main-

TABLE 6

Atmospheric pressure, oxygen No. 19 and No. 20, 9 per cent; No. 21, 8 per cent

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			per cent			per cent
Rabbit No. 19...	1580	1390	—190	7.1	7.1	0	13.8	14.5	+5.1
20...	1660	1107	—553	7.2	8.3	+15.3	14.1	16.9	+20.0
21...	1150	1165	+15	6.9	8.3	+20.3	13.8	16.9	+22.5
Average percentage blood change						+11.9			+15.9

TABLE 7

Atmospheric pressure, oxygen 6 per cent

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			per cent			per cent
Rabbit No. 27...	840	630	—210	6.6	8.2	+24.2	12.8	18.6	+45.3
28...	850	560	—290	5.9	6.6	+11.9	11.9	14.9	+25.2
Average percentage blood change						+18.0			+35.2

TABLE 8

Atmospheric pressure, oxygen 10 per cent. Showing progressive blood changes during experiment and the return to normal following experiment

ANIMAL	WEIGHT	ERYTHROCYTES MILLION PER CMM.	HAEMOGLOBIN GR. PER 100 CC. BLOOD
Dog No. 1			
Before experiment.....	2320	6.6	11.8
2 day run.....	2380	6.6 (0)	12.4 (+5.1%)
4 day run.....	2420	5.9 (-10.6%)	11.3 (-4.2%)
12 day run.....	2460	7.6 (+15.2%)	13.7 (+16.1%)
8 days after experiment		7.1 (+7.6%)	13.2 (+11.9%)
29 days after experiment		5.9 (-10.6%)	11.6 (-1.7%)
Dog No. 2			
Before experiment.....	3700	6.1	10.2
2 day run.....	3820	6.5 (+6.5%)	12.6 (+23.5%)
4 day run.....	3780	6.5 (+6.5%)	12.8 (+25.3%)
12 day run.....	3520	8.2 (+34.4%)	14.3 (+40.2%)
8 days after experiment		7.7 (+26.2%)	13.6 (+33.3%)
29 days after experiment		6.4 (+4.9%)	11.7 (+14.7%)
40 days after experiment		6.1 (0)	10.6 (+3.9%)
Rabbit No. 15			
Before experiment.....	1050	5.4	10.6
3 day run.....		5.9 (+9.3%)	13.3 (+25.5%)
6 day run.....	1100	6.6 (+22.2%)	16.8 (+58.5%)
11 day run.....	1130	5.7 (+5.6%)	15.9 (+50.0%)
Rabbit No. 16			
Before experiment.....	1010	6.0	11.2
3 day run.....		6.1 (+1.7%)	12.3 (+9.8%)
6 day run.....	1095	7.0 (+16.7%)	15.0 (+33.9%)
11 day run.....	1150	6.9 (+15.0%)	15.3 (+36.6%)

TABLE 9

Atmospheric pressure, oxygen 6 per cent. Showing effect of 2 and 7 day exposure to the atmosphere

ANIMAL	WEIGHT	ERYTHROCYTES MILLION PER CMM.	HAEMOGLOBIN GR. PER 100 CC. BLOOD
Rabbit No. 27			
Before experiment.....	840	6.6	12.8
2 day run.....	720	7.2 (+9.1%)	14.7 (+14.8%)
7 day run.....	630	8.2 (+24.2%)	18.6 (+45.3%)
Rabbit No. 28			
Before experiment	850	5.9	11.9
2 day run.....	750	6.2 (+5.1%)	13.8 (+16.0%)
7 day run.....	560	6.6 (+11.9%)	14.9 (+25.2%)

TABLE 10

Atmospheric pressure, oxygen 10 per cent. Showing the persistence of blood effects after experiment

ANIMAL	WEIGHT	ERYTHROCYTES MILLION PER CMM.	HAEMOGLOBIN GR. PER 100 CC. BLOOD
Rabbit No. 7			
Before experiment.....	1620	7.0	12.4
7 day run.....	1550	7.6 (+8.6%)	17.2 (+38.7%)
1 day after experiment		8.0 (+14.3%)	14.8 (+19.4%)
2 days after experiment			15.4 (+24.2%)
3 days after experiment			16.1 (+29.8%)
18 days after experiment			16.2 (+30.6%)
Rabbit No. 8			
Before experiment	1650	6.4	16.9
7 day run.....	1620	8.5 (+32.8%)	19.5 (+15.4%)
1 day after experiment		8.3 (+29.7%)	19.8 (+17.2%)
6 days after experiment		8.5 (+32.8%)	19.5 (+15.4%)
Rabbit No. 9			
Before experiment.....	970	5.8	11.6
7 day run.....	1060	7.5 (+29.3%)	15.7 (+35.3%)
2 days after experiment		7.7 (+32.8%)	16.7 (+44.0%)
10 days after experiment		7.4 (+27.6%)	15.3 (+31.9%)
33 days after experiment		6.0 (+3.4%)	11.8 (+1.0%)
Rabbit No. 10			
Before experiment.....	935	6.5	13.7
7 day run.....	1070	7.3 (+12.3%)	16.4 (+19.7%)
2 days after experiment		7.2 (+10.8%)	15.8 (+15.3%)
10 days after experiment		7.6 (+16.9%)	16.8 (+22.6%)
33 days after experiment	1340	7.5 (+15.4%)	15.8 (+15.3%)
Rabbit No. 11			
Before experiment.....	898	5.8	11.5
7 day run.....	973	7.3 (+25.9%)	17.0 (+47.8%)
2 days after experiment		7.2 (+24.1%)	14.7 (+27.8%)
10 days after experiment		6.9 (+19.0%)	14.8 (+28.7%)
33 days after experiment	1250	6.9 (+19.0%)	14.5 (+26.1%)
Rabbit No. 12			
Before experiment.....	1200	6.9	14.6
7 day run.....	1220	7.8 (+13.0%)	16.6 (+13.7%)
5 days after experiment	1280	6.9 (0)	17.4 (+19.2%)
Rabbit No. 13			
Before experiment.....	1280	6.8	12.0
7 day run.....	1300	7.1 (+4.4%)	16.2 (+35.0%)
5 days after experiment	1300	6.7 (-1.5%)	17.3 (+44.2%)
Rabbit No. 14			
Before experiment.....	1120	6.9	13.3
7 day run.....	1210	7.2 (+4.3%)	15.6 (+17.3%)
5 days after experiment	1320	7.1 (+2.9%)	17.2 (+29.3%)

tain. The ventilation of the reduced pressure chamber was from 22 to 24 liters per minute. At this rate of ventilation with two rabbits in the chamber (this number was always employed) the oxygen was always 20.5 per cent and the carbon dioxide 0.2 per cent.

Results. The results can be presented most satisfactorily in tabular form. The experiments are divided into two large groups, (1) those at atmospheric pressure and (2) those at reduced pressure. In these two sets of experiments we have attempted to work at approximately the same pressure of oxygen. We shall first present the larger group of

TABLE 11

Atmospheric pressure, oxygen No. 19 and No. 20, 9 per cent; No. 21, 8 per cent. Showing effect of 7 and 14 day exposures and in No. 21 the post-experimental effect

ANIMAL	WEIGHT	* ERYTHROCYTES MILLION PER CMM.	HAEMOGLOBIN GR. PER 100 CC. BLOOD
Rabbit No. 19			
Before experiment.....	1580	7.1	13.8
7 day run.....		7.1 (0)	14.5 (+5.1%)
14 day run.....	1390	7.5 (+5.6%)	16.5 (+19.6%)
Rabbit No. 20			
Before experiment.....	1660	7.2	14.1
7 day run.....		8.3 (+15.3%)	16.9 (+19.9%)
14 day run.....	1107	9.0 (+25.0%)	18.1 (+28.4%)
Rabbit No. 21			
Before experiment.....	1150	6.9	13.8
7 day run.....	1165	8.3 (+20.3%)	16.9 (+22.5%)
14 day run.....	1090	7.9 (+14.5%)	17.0 (+23.2%)
3 days after experiment	1105	8.8 (+27.5%)	19.0 (+37.7%)

TABLE 12

Experiments with white rats. Atmospheric pressure oxygen 10 per cent

Duration of experiments—three weeks

CONTROL RATS IN 20.8 PER CENT OXYGEN		RATS IN 10 PER CENT OXYGEN	
<i>Erythrocytes</i>		<i>Erythrocytes</i>	
No. 1.....	9.1	No. 5.....	11.4
No. 2.....	7.4	No. 6.....	10.7
No. 3.....	7.8	No. 7.....	10.1
No. 4.....	9.8	No. 8.....	11.3
Average.....	8.5	Average.....	10.9

TABLE 12—Continued.

CONTROL RATS IN 20.8 PER CENT OXYGEN		RATS IN 10 PER CENT OXYGEN	
<i>Haemoglobin</i>		<i>Haemoglobin</i>	
No. 1.....	17.1	No. 5.....	21.2
No. 2.....	13.7	No. 6.....	19.3
No. 3.....	17.0	No. 7.....	19.8
No. 4.....	13.6	No. 8.....	21.5
Average.....	15.8 g. per 100 cc. blood	Average.....	20.5 g. per 100 cc. blood

Erythrocytes increased 2.4 million or 28.2 per cent.

Haemoglobin increased 4.7 g. per 100 cc. blood or 29.7 per cent.

Control rats (20.8 per cent oxygen).

No. 11. 7.3 gr. haemoglobin per kilo body weight.

No. 12. 7.8 gr. haemoglobin per kilo body weight.

Average, 7.55 gr. per kilo.

Rats after three weeks in 10 per cent oxygen.

No. 9. 12.5 gr. haemoglobin per kilo body weight.

No. 10. 9.1 gr. haemoglobin per kilo body weight.

Average, 10.8 gr. per kilo.

Increase, 3.25 gr. per kilo or 43 per cent.

TABLE 13

Atmospheric pressure. Showing effect of high content of carbon dioxide with the normal oxygen content (20.5 per cent) of atmosphere. Carbon dioxide No. 105 and No. 106 = 0.5 per cent, No. 17 and No. 18. Carbon dioxide = 1 per cent

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			<i>grams</i>			<i>per cent</i>			<i>per cent</i>
Rabbit No. 105..	1520	1290	—230	6.5	6.9	+6.2	13.4	13.7	+2.2
No. 106..	1460	1360	—100	6.2	6.8	+9.7	13	14.9	+14.6
Average percentage blood change at 0.5% CO ₂ ...+8.0									+8.4
Rabbit No. 17...	1300	1410	+110	6.5	7.1	+9.2	12.8	15.0	+17.2
No. 18...	930	940	+10	6.1	6.7	+9.8	11.2	12.6	+12.5
Average percentage blood change at 1% CO ₂ ...+9.5									+14.8

experiments, i.e., those at atmospheric pressure. This group of experiments is further subdivided according to the animals used and according to the length of the experiments. Tables are also given showing the post-experimental effects of diminished oxygen pressure and the time required to return to normal. For the purpose of illustrating these various points it will be seen that the same experiments may be recorded in different tables.

Tables 1 to 13 record experiments at atmospheric pressure.

Total haemoglobin determinations were made on four rats of this series.

Tables 14 to 20 record experiments at reduced barometric pressure.

TABLE 14

Reduced barometric pressure. Pressure = 593 mm. Hg. Corresponding oxygen tension, 17 per cent of an atmosphere, equivalent altitude, 2020 m.

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			per cent			per cent
Rabbit No. 33...	1800	1560	-240	7.4	7.0	-5.4	13.2	13.8	+4.5
No. 34...	1700	1440	-260	7.2	8.1	+12.5	15.5	14.5	-6.5
Average percentage blood change.....									+3.5

TABLE 15

Reduced barometric pressure. Pressure = 495 mm. Hg. Corresponding oxygen tension, 14 per cent of an atmosphere, equivalent altitude 3,500 m.

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			per cent			per cent
Rabbit No. 33...	1560	1610	+50	7.0	7.2	+2.9	13.8	14.1	+2.2
No. 34..	1440	1520	+80	8.1	8.1	0	14.5	14.9	+2.8
No. 35...	1760	1700	-60	5.0	5.5	+10.0	11.5	12.6	+9.6
No. 36...	1690	1640	-50	5.6	4.6	-17.9	9.7	9.7	0
No. 44...	1520	1440	-80	5.8	6.7	+15.5	11.0	13.3	+20.9
No. 45...	1420	1410	-10	5.3	6.5	+22.6	12.1	13.5	+11.6
Average percentage blood change.....									+7.8

DISCUSSION OF RESULTS

The results shown in tables 1 to 6 clearly prove that great increases in erythrocytes occur when dogs, rabbits or rats are kept in an atmosphere of low oxygen concentration even at atmospheric pressure. When the atmosphere of the chamber contained the normal concentration of oxygen (20.8 per cent) or even 16 per cent oxygen the changes in the blood count were insignificant. When the oxygen is reduced to 14 per cent marked increases in the blood count occur within one week. The increases noted at 14 per cent and 12 per cent oxygen in our

TABLE 16

Reduced barometric pressure. Pressure 422 mm. Hg. Corresponding oxygen tension 12 per cent of an atmosphere, equivalent altitude, 4730 m.

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			per cent			per cent
Rabbit No. 37...	1860	1780	-80	5.2	6.0	+15.4	8.9	12.6	+41.6
No. 38...	1760	1640	-120	5.3	6.8	+28.3	8.3	14.0	+68.7
Average percentage blood change									+55.1

TABLE 17

Reduced barometric pressure. Pressure 352 mm. Hg. Corresponding oxygen tension, 10 per cent of an atmosphere, equivalent altitude, 6060 m.

Duration of experiment No. 48 and No. 49 four days; No. 50
and No. 51 five days

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			per cent			per cent
Rabbit No. 48...	1950	1640	-310	4.6	4.5	-2.2	6.9	9.8	+42.0
No. 49...	1830	1620	-210	6.3	6.5	+3.2	13.2	15.0	+13.6
No. 50...	1370	1330	-40	7.5	8.7	+16.0	14.9	19.8	+32.9
No. 51...	1420	1380	-40	7.5	8.1	+8.0	14.5	17.3	+19.3
Average percentage blood change.....									+27.0

series are practically identical. At 10 per cent oxygen the increases reach the maximum, 18 per cent increase in erythrocytes and 26.7 per cent increase in haemoglobin. The number of experiments at 9 per cent, 8 per cent, and 6 per cent oxygen are too few in number to conclude what the average increase in a large number of animals would be. The variations in individuals in their reaction to atmospheres low in oxygen is so great that large numbers of experiments must be performed in order to arrive at a reliable average figure for a given oxygen concentration. It is to be noted that anaemic animals react more markedly to a

TABLE 18

Reduced barometric pressure. Pressure = 352 mm. Hg. Corresponding oxygen tension 10 per cent of an atmosphere, equivalent altitude, 6060 m.

Duration of experiment—one day

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			per cent			per cent
Rabbit No. 46...	1800	1720	-80	5.9	6.6	+11.9	13.6	14.1	+3.7
No. 47...	2020	1910	-110	6.3	6.5	+3.2	13.8	13.9	+0.7
No. 48...	1950	1760	-190	4.6	4.6	0	6.9	7.6	+10.0
No. 49...	1830	1700	-130	6.3	6.3	0	13.2	13.4	+1.5
No. 50...	1370	1370	0	7.5	7.4	-1.3	14.9	14.9	0
No. 51...	1420	1440	+20	7.5	7.7	+2.7	14.5	14.7	+1.4
Average percentage blood change.....									+2.9

TABLE 19

Reduced barometric pressure. Pressure = 352 mm. Hg. Corresponding oxygen tension 10 per cent of an atmosphere equivalent altitude 6060 m.

Duration of experiment—two days

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			per cent			per cent
Rabbit No. 46...	1800	1720	-80	5.9	6.5	+10.2	13.6	14.1	+3.7
No. 47...	2020	1890	-130	6.3	6.8	+7.9	13.8	14.9	+8.0
No. 48...	1950	1690	-260	4.6	4.3	-6.5	6.9	7.6	+10.0
No. 49...	1830	1660	-170	6.3	6.2	-1.6	13.2	13.7	+3.8
No. 50...	1370	1320	-50	7.5	7.5	0	14.9	15.1	+1.3
No. 51...	1420	1420	0	7.5	7.5	0	14.5	15.6	+7.6
Average percentage blood change.....									+5.7

reduction in the partial pressure of oxygen, an observation in keeping with the results of David.⁵¹ It would seem, however, from the limited number of experiments performed that the maximum increases in the blood count occur when the oxygen concentration is near 10 per cent.

TABLE 20

Reduced barometric pressure. Pressure 352 mm. Hg. Corresponding oxygen tension 10 per cent of an atmosphere, equivalent altitude, 8060 m.

ANIMAL	WEIGHT	ERYTHROCYTES MILLION PER CMM.	HAEMOGLOBIN GR. PER 100 CC. BLOOD
Rabbit No. 46			
Before experiment.....	1800	5.9	13.6
1 day run.....	1720	6.6 (+11.9%)	14.1 (+3.7%)
2 day run.....	1720	6.5 (+10.2%)	14.1 (+3.7%)
Rabbit No. 47			
Before experiment.....	2020	6.3	13.8
1 day run.....	1910	6.5 (+3.2%)	13.9 (+0.7%)
2 day run.....	1890	6.8 (+7.9%)	14.9 (+8.0%)
Rabbit No. 48			
Before experiment.....	1950	4.6	6.9
1 day run.....	1760	4.6 (0)	7.6 (+10.0%)
2 day run.....	1690	4.3 (-6.5%)	7.6 (+10.0%)
4 day run.....	1640	4.5 (-2.2%)	9.8 (+42.0%)
Rabbit No. 49			
Before experiment.....	1830	6.3	13.2
1 day run.....	1700	6.3 (0)	13.4 (+1.5%)
2 day run.....	1660	6.2 (-1.6%)	13.7 (+3.8%)
4 day run.....	1620	6.5 (+3.2%)	15.0 (+13.6%)
Rabbit No. 50			
Before experiment.....	1370	7.5	14.9
1 day run.....	1370	7.4 (-1.3%)	14.9 (0)
2 day run.....	1320	7.5 (0)	15.1 (+1.3%)
5 day run.....	1330	8.7 (+16.0%)	19.8 (+32.9%)
Rabbit No. 51			
Before experiment.....	1420	7.5	14.5
1 day run.....	1440	7.7 (+2.7%)	14.7 (+1.4%)
2 day run.....	1420	7.5 (0)	15.6 (+7.6%)
5 day run.....	1380	8.1 (+8.0%)	17.3 (+19.3%)

It is very interesting that increases in the blood count occur even with oxygen concentrations at the lowest level at which it is possible to keep an animal continuously for a week in fairly good condition. It has been shown previously in this laboratory that the stimulation of the medul-

⁵¹ David Deutsch. Arch. f. klin. Med., 1913, cix, 129.

lary centers by decreased oxygen fixation passes into depression and paralysis when oxygen fixation falls below a certain level. The work here reported indicates that the bone marrow differs in this regard from the medullary centers. Since approximately a week is required for stimulation of the bone marrow to become evident it would seem that the nervous system would succumb before oxygen fixation by the bone marrow could be reduced to the point of depression. The relative effect of different concentrations of oxygen at atmospheric pressure is shown in the following table:

TABLE 21

OXYGEN	NUMBER OF EXPERIMENTS	BLOOD CHANGE	
		Erythrocytes	Haemoglobin
<i>per cent</i>		<i>per cent</i>	<i>per cent</i>
20	7	2.7	9.7
16	4	4.6	-1.9
14	8	15.2	17.4
12	5	14.4	16.8
10	15	18.5	26.7
(9, 8, and 6)	5	14.3	29.0

The results prove beyond doubt that a decrease in the partial pressure of the oxygen of the respired air causes an increase in the erythrocytes and haemoglobin per unit volume of blood and that the increase occurs even at atmospheric pressure under conditions which eliminate all of the physical effects of decreased barometric pressure and high mountain climates.

If a sufficiently large number of experiments had been performed at each oxygen concentration the irregularities in the above table would in all probability have disappeared. In this case it probably would have been noted that the effect of lowering the oxygen tension would have increased more or less regularly after 14 per cent oxygen had been reached until the optimum, about 10 per cent, had been attained. This would have involved a large expenditure of time and energy which in the presence of the above results was not deemed necessary.

Tables 14 to 17 show the effect of corresponding partial pressures of oxygen obtained by reducing the barometric pressure. The results are summarized in the following table:

TABLE 22

BAROMETRIC PRESSURE MM. HG.	NUMBER OF EXPERIMENTS	PARTIAL PRESSURE OF OXYGEN IN PER CENT OF AN ATMOSPHERE	BLOOD CHANGE	
			Erythrocytes	Haemoglobin
593	2	17	+3.5	-1.0
495	6	14	+5.6	+7.8
422	2	12	+21.8	+55.1
352*	4*	10*	+6.2*	+27.0*

* These experiments only lasted four to five days whereas the others were of a week's duration.

It will be noted on examining tables 14 to 17 that the average figures given above were obtained from relatively few experiments and therefore merely indicate the general trend of what average results might have been obtained if a larger number of individuals had been used. Thus in the work at 12 per cent oxygen only two animals were used. We regard it as highly improbable that in a large number of experiments animals would show as marked an increase in the blood count at 12 per cent as at 10 per cent oxygen. Unfortunately the experiments at 10 per cent oxygen were not allowed to proceed a week as in the other cases but were interrupted at the end of four or five days. This was due to the fact that at the time the experiments were performed other phases of the subject were under consideration. On comparing tables 20 and 21 it will be seen that the increase in the blood count at reduced oxygen tension occurs whether the partial pressure of the oxygen is reduced by diluting the oxygen at atmospheric pressure with the inert gases of the atmosphere or by reducing the barometric pressure. The general trend of the results in both cases is the same and we believe that if a sufficiently large number of observations were made there would be no difference.

The question then presents itself whether the increased blood counts noted are to be attributed to concentration of the blood, i.e., a reduction in the total blood volume, to changes in the distribution of the erythrocytes or to increased production of erythrocytes and haemoglobin by the bone marrow. The fact that the increase in blood count is noted at atmospheric pressure when the partial oxygen pressure is lowered even with a humidity which is equal to or greater than that of the normal atmosphere speaks strongly against increased evaporation of water as the mechanism by which any increase in the concentration of the blood could occur. Furthermore, a study of the weight of the

animals before and after experiments proves conclusively that the increased blood counts per unit volume of blood cannot be due to increased evaporation of water from the animals. In many cases the animals gained in weight during the experiment where marked increase in the count was noted. In other cases the animals showed marked loss of weight without any change in the count. The blood changes were entirely independent of any changes in the weight of the animals, as the tables definitely show.

In order to determine whether there was an alteration in the distribution of the erythrocytes and plasma in the large and small vessels as suggested by Foa³² we have made counts of blood obtained from the marginal ear vein and then of blood from the carotid and heart. In all cases the counts were the same within the limits of experimental error. The only probable explanation remaining is that the increased blood counts are due to increased activity of the bone marrow. As further evidence in favor of this view we present the following observations: (1) We found in our work on the white rat, Table 12, that the animals kept for three weeks in an atmosphere of 10 per cent oxygen at atmospheric pressure possess on the average 43 per cent more haemoglobin per kilo body weight than the animals kept in the ordinary atmosphere. These findings are in keeping with the results of Jaquet and Suter³³ and Abderhalden³⁴ who made similar determinations. (2) Blood smears from animals showing increased blood counts after exposure to atmospheres poor in oxygen when stained with Jenner stain show a number of basophilic erythrocytes. This staining reaction is characteristic of newly formed erythrocytes. Many of these basophilic erythrocytes were abnormally large especially in those animals which showed a marked increase in the haemoglobin without very much change in the number of erythrocytes. This probably explains the increase in the amount of haemoglobin per corpuscle in certain cases. (3) Examination of the bone marrow showed macroscopically a disappearance of the fat and an extension of the red marrow and microscopically a marked hyperplasia of the erythrocyte forming centers and an equally marked dilatation of the capillaries and small vessels of the marrow which were engorged with blood. The picture is practically identi-

³² Foa: *Laborat. scient. internat. du Mont Rosa. Trav. de l'année 1903*, Turin 1904 cited from Zuntz, Loewy, Müller and Caspari, "Hohenklime und Bergwanderungen," 1906.

³³ Jaquet and Suter: *Correspondenzblt. f. Schweizer Aerzte*, 1898, xxviii, 104.

³⁴ Abderhalden: *Zeitsch. f. Biol.*, 1902, xliii, 123.

cal with that seen after haemorrhage except for the engorgement of the vessels which is not seen in the latter condition. We are greatly indebted to Dr. C. H. Bunting for the microscopical examination of the blood smears, bone marrow and other organs of our animals and we desire herewith to extend our thanks to him and to acknowledge his valuable assistance.

The relation between the increase in the number of erythrocytes and haemoglobin noted in our experiments is interesting. Oliver³⁵ working at Arosa and Davos found that the erythrocytes increase 10 per cent and the haemoglobin 5 per cent. Roemisch³⁶ found at Arosa that the increase in the haemoglobin was about half as great as the increase in erythrocytes. Van Voornveld³⁷ states that most investigators agree that the erythrocytes increase more rapidly than the haemoglobin. Abderhalden found that the increase in erythrocytes is always proportional to the increase in haemoglobin. Schaumann and Rosenqvist³⁸ find that the increase in haemoglobin lags behind that in erythrocytes. Eggers³⁹ found in two people there occurred a much smaller increase in erythrocytes than in haemoglobin. Bürker and his co-workers⁴⁰ likewise found that in two persons investigated the haemoglobin showed a greater increase than the erythrocytes. In their work on artificial pneumothorax, Bürker, Ederle and Kircher⁴¹ found that the erythrocyte count increased more rapidly and showed a greater increase than the haemoglobin in the one animal of which they publish the details of the experiment. Nasmith and Harrison⁴² state that animals chronically poisoned with carbon monoxide show a relatively greater increase in erythrocytes than in haemoglobin.

Under the conditions obtaining in our work the haemoglobin showed on the average a much greater increase than the erythrocytes in response to decreased partial pressure of oxygen both at atmospheric and at reduced barometric pressure. There are, however, many individual cases where the increase in the erythrocytes is greater than that of the haemoglobin as may be seen from the tables. These cases are in the

³⁵ Oliver: "A contribution to the study of the blood and blood pressure," London, 1901.

³⁶ Roemisch: Cited from Van Voornveld, q. v.

³⁷ Van Voornveld: Arch. f. d. gesamt. Physiol., 1902, xcii, 1.

³⁸ Schaumann and Rosenqvist: Zeitschr. f. klin. Med., 1898, xxxv, 126, 315.

³⁹ Eggers: Arch. f. exp. Path. u. Pharm., 1897, xxxix, 426.

⁴⁰ Bürker, Jooss, Moll and Neumann: Zeitsch. f. Biol., 1913, lxi, 379.

⁴¹ Bürker, Ederle and Kircher: Zentralblt. f. Physiol., 1913, xxvii, p. 623.

⁴² Loc. cit.

minority, however. In some cases as in Rabbit 7, table 5, the increase in haemoglobin was so great compared with the increase in the erythrocytes that we examined the plasma for haemoglobin, but never found any haemoglobin outside of the corpuscles. Therefore in most of our experiments there was found an increase in the amount of haemoglobin per corpuscle. The greatest increase in the haemoglobin per corpuscle in the experiments at atmospheric pressure was 30 per cent, noted in Rabbit 15, table 5. The greatest decrease in haemoglobin per corpuscle was 20 per cent noted in Rabbit 39, table 3. The effect of reduced oxygen pressure on the color index is therefore very variable.

The time required for the increased blood count to develop is of much interest. Investigations of the effect of high altitude on the blood count do not agree entirely as to the time of onset of the blood change. Ehrlich and Lazarus⁴³ state that the increase occurs immediately on reaching a place of considerable altitude. Oliver⁴⁴ states that the increase occurs soon after arrival at high altitude, being apparent within twenty-four hours. Abderhalden⁴⁵ found that the erythrocytes and haemoglobin increased within a few hours. Douglas, Haldane, Henderson and Schneider⁴⁶ found that erythrocytes and haemoglobin increase during the first few days and continue to increase for several weeks on Pike's Peak. Schneider and Havens⁴⁷ find that a rapid increase in erythrocytes and haemoglobin in the peripheral vessels occurs during the first two to four days of residence at high altitude. Our results on this point at atmospheric pressure are shown in tables 8 and 9 and at reduced barometric pressure in tables 18, 19, and 20. From tables 8 and 9 it will be seen that all of the animals except Dog 1 and Rabbit 16 show definite increase in the blood counts at the time of the first observations, viz., after two or three days. On the other hand, at low barometric pressure corresponding to 10 per cent oxygen, observations on six rabbits showed no increase after twenty-four or forty-eight hours' exposure to the atmosphere. Our observations were not made on a sufficiently large number of animals to admit of generalization. In no case, however, were the maximum counts obtained in less than one week.

⁴³ Ehrlich and Lazarus: *Anaemia*, Nothnagel's Encyclopedia, Philadelphia and London, 1905, p. 22.

⁴⁴ Loc. cit.

⁴⁵ Loc. cit.

⁴⁶ Loc. cit.

⁴⁷ Schneider and Havens: *This Journal*, 1915, xxxvi, 380.

A few experiments were performed to determine whether a longer exposure to the atmospheres poor in oxygen would result in further increasing the blood count. These experiments in which animals were exposed for periods longer than one week to atmospheres low in oxygen indicated that the maximal count is not reached within one week. (Table 11.) This is in agreement with the results of work at high altitude.

In regard to the post-experimental blood changes our results are also in perfect accord with the work of many investigators on the effect of high altitude. Following the removal of animals from the respiratory chamber into the ordinary atmosphere there was often noted a further increase in the blood counts during the first and second day (Table 10). Following this period the erythrocytes and haemoglobin very gradually return to normal often requiring more than a month to reach the level obtaining before the exposure to atmospheres of low oxygen. This slow return to the normal has been noted by all workers at high altitude and the fact that the same effects are noted at normal barometric pressure when the partial pressure of oxygen alone is reduced is striking evidence of the identity of the blood changes under the two conditions and leads to the conclusion that the blood changes noted at high altitude are due essentially to the low partial pressure of oxygen.

Mechanism of the stimulation of the bone marrow. All of the facts presented in the foregoing part of this communication lead to the conclusion that the increase in erythrocytes and haemoglobin at high altitude and in atmospheres of low partial pressure of oxygen whether at atmospheric pressure or at reduced barometric pressure is due to stimulation of the bone marrow. The question of the mechanism of the stimulation then presents itself. Bert looked upon the increase in the oxygen capacity of the blood at high altitude as constituting an important factor in acclimatization to an atmosphere of reduced oxygen tension. Viault, Müntz, and other workers agreed with Bert. The statement that it is the principal factor in acclimatization is not an explanation of the mechanism by which it occurs. A review of the literature since the early work shows clearly that investigators were loathe to accept the increase in the oxygen capacity of the blood at high altitude as a fact or in the face of excellent evidence they sought to explain it as purely a relative increase without any physiological significance. What was the reason for this attitude on the part of investigators? It would seem that the statement, "when the oxygen tension of the air is decreased the oxygen capacity of the blood is in-

creased" was regarded as too teleological to be true. Miescher,⁴⁸ however, accepted the proposition as proved and sought to interpret the mechanism of the reaction. He believed that there exists in the bone marrow normally a condition of relative oxygen want which maintains the bone marrow in a condition of activity so that erythrocytes and haemoglobin are being produced constantly at a certain rate. He believed that any further reduction of the oxygen supply to the bone marrow increases this activity. He sought other biological parallels to this stimulation by oxygen want and referred to the increased production of alcohol by yeast under anaerobic conditions. Our views agree closely with those of Miescher. We feel that the parallelism with the activity of yeast which Miescher tried to draw was not the happiest one which could be found. It had been long known that the respiratory center responded with increased functional activity to oxygen want. Furthermore, oxygen want or any other method of reducing oxygen fixation has since been shown to stimulate the respiratory, vaso-constrictor and cardio-inhibitory centers, and also the motor cortex. We have therefore abundant analogy for the stimulation of the bone marrow by decreased oxygen supply to the bone marrow. Stimulation by decreased oxygen fixation and the mechanism by which it may be conceived to be brought about have been discussed by Gasser and Loevenhart⁴⁹ and by Loevenhart.⁵⁰ The external respiration, the circulation and the bone marrow are the three physiological mechanisms which maintain the chemical environment essential for tissue respiration. The respiratory movements are controlled by the respiratory center, the oxygen capacity of the blood is controlled certainly in large part by the activity of the bone marrow. It is therefore interesting to compare the reactions of the respiratory center and the bone marrow to alterations in the oxygen and carbon dioxide content of the blood as well as to other conditions altering oxidations in the body. Gasser and Loevenhart have shown that the injection of threshold doses of sodium cyanide or carbon monoxide affect the respiratory center before any other function is measurably affected. This indicated that the cells of the respiratory center are more sensitive to decreased oxygen fixation than any other cells in the body so far as could be determined. It was shown by Haldane and Priestley⁵¹ that the oxygen of the respired air must fall

⁴⁸ Miescher: *Correspondenzbl. f. Schweizer Aerzte*, 1893, xxiii, 809.

⁴⁹ Gasser and Loevenhart: *Loc. cit.*

⁵⁰ Loevenhart: *Arch. Internal Med.*, 1915, xv, 1059.

⁵¹ Haldane and Priestly: *Journ. Physiol.*, 1905, xxxii, 225.

to 13 per cent of an atmosphere before the respiratory center is stimulated. It will be seen from our work that we have observed no stimulation of the bone marrow when the oxygen of the respired air falls to 16 per cent. At 14 per cent oxygen we noted stimulation of the bone marrow. At high altitude, however, a decrease of the oxygen pressure of much smaller magnitude has been found to stimulate the bone marrow. It may be that in these cases other factors than oxygen tension play a rôle since in our experiments both at atmospheric and at reduced barometric pressure stimulation was not observed until the oxygen tension reached 14 per cent of an atmosphere. It would seem, therefore, that the bone marrow is perhaps a trifle more sensitive to a decrease in the oxygen supply than the respiratory center. This may be due to the fact that the respiratory center must respond quickly or not at all to decreased oxygen fixation and that it adapts itself more quickly to the new conditions for oxidation than does the bone marrow. In fact it would seem that the bone marrow has no power to adapt itself to decreased oxygen supply since the increased rate of erythrocyte and haemoglobin formation continue indefinitely for a given oxygen tension. It would seem as though the oxygen of the respired air would have to fall to near 14 per cent before the percentage saturation of the haemoglobin with oxygen is appreciably reduced. The respiratory center or the bone marrow could respond only to a decrease in the oxygen of the respired air if this were sufficient to alter their supply of oxygen. It would seem that while the bone marrow is a trifle more sensitive than the respiratory center, the two are however approximately equally sensitive to a decreased supply of oxygen. Grove and Loevenhart and Gasser and Loevenhart have shown that if oxygen fixation by the respiratory center is reduced below a certain level there results a temporary depression or paralysis of the respiratory center. We have made an attempt to determine whether the bone marrow may be likewise depressed. Exposure of animals for a week to an atmosphere of 6 per cent oxygen failed to show any depression of the bone marrow. In fact it was stimulated. It seemed impractical therefore to demonstrate depression of the bone marrow by decreasing oxygen fixation. At such low concentrations of oxygen the animals refuse to eat and conditions became too abnormal in experiments of a week's duration to admit of a clear cut interpretation and the attempt was abandoned. A comparison of the reaction of the respiratory center and the bone marrow to an increase in the carbon dioxide is likewise of considerable interest. It is our belief that the mechanism of this stimulation is

to be found in the acid properties of carbon dioxide and its consequent power to decrease oxygen fixation. In terms of this theory we would say that the velocity of oxygen fixation (R-processes) of the respiratory center is extremely sensitive to carbon dioxide or in other words, to the hydrogen ion concentration. We performed a few experiments (table 13) to determine how sensitive the bone marrow is to an increase in the carbon dioxide. We found definite but slight stimulation of the bone marrow by concentrations of 0.5 per cent to 1 per cent carbon dioxide in the respired air. The bone marrow is therefore far less sensitive to carbon dioxide than the respiratory center. Expressed in terms of our theory therefore, the velocity of oxygen fixation (R-processes) is relatively far less sensitive to carbon dioxide or to the hydrogen ion concentration in case of the bone marrow than in that of the respiratory center. The relative effectiveness of oxygen want and excess of carbon dioxide in altering the processes of oxidation and causing stimulation not only varies in the different tissues of the same individual or species but probably also varies in the same tissue of different species. Thus it would appear that the respiratory center of diving birds is not stimulated by an increase of carbon dioxide, but responds readily to oxygen want. In all cases we believe that the stimulation by oxygen want, excess of carbon dioxide, hydrocyanic acid and many other means is to be attributed to a primary decrease in oxygen fixation or in other words to a primary decrease in the velocity of the R-processes, those oxidative processes which are characteristic of rest and recuperation. The R-processes of different tissues vary greatly in their relative sensitiveness to decreased supply of oxygen and excess of carbon dioxide. The work here presented is in keeping with the theoretical views previously published from this laboratory relative to the A and R processes and the inverse relationship existing between oxygen fixation and functional activity.

CÓNCLUSIONS AND SUMMARY

1. A decrease in the oxygen tension of the respired air obtained by decreasing the oxygen concentration at atmospheric pressure or by reducing the barometric pressure stimulates the bone marrow and increases the erythrocytes and haemoglobin in the circulating blood in rabbits, white rats and dogs. From five to seven days is required for the increase in the blood count to become very marked but the maximum increase requires a longer exposure.

2. The increase in the erythrocytes and haemoglobin is absolute and not relative. We have been able to increase the total haemoglobin per kilo in rats 43 per cent.

3. In order to produce these effects the oxygen pressure in the respired air must be reduced at least to approximately 14 per cent of an atmosphere.

4. The optimum oxygen pressure for increasing the oxygen capacity of the blood is apparently not far from 10 per cent of an atmosphere.

5. We have not been able to produce depression of the bone marrow by decreasing the oxygen supply. Stimulation results even when the oxygen pressure falls to 6 per cent of an atmosphere.

6. It is possible to stimulate the bone marrow to a certain extent by increasing the carbon dioxide tension of the respired air but it is not an efficient stimulus.

7. A comparison of the respiratory center and the bone marrow in their reaction to a decreased supply of oxygen and an excess of carbon dioxide shows that they are practically equally sensitive to oxygen want but that the respiratory center is far more sensitive to carbon dioxide than is the bone marrow.

8. Our work indicates that the increase in erythrocytes and haemoglobin noted by practically all workers at high altitudes is due largely if not entirely to the decreased partial pressure of oxygen at high altitude, but the very rapid increases in the blood counts noted in man at high altitude and also the increases noted at comparatively very slight elevations is not explained by our work.

9. The physiological significance of the increase in the oxygen capacity of the blood when an atmosphere of low oxygen tension is respired is sufficiently obvious to require no comment.

10. The stimulation shown by the bone marrow in response to decreased oxygen supply is by no means unique. Similar reactions are to be seen in the respiratory, vaso-constrictor and cardio-inhibitory centers and the mechanism is in all cases probably identical. The work is entirely in keeping with the views previously published from this laboratory in regard to the probable mechanism of stimulation by oxygen want that there is inverse relation between changes in the rate of oxygen fixation and functional activity.

THE STIMULATION OF THE HYPOPHYSIS IN DOGS

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A. INTRODUCTION AND LITERATURE

Attempts to produce glycosuria by the injections of extracts of the hypothesis were reported by Borchardt¹ in 1908. He found that subcutaneous injections of 2 to 4 glands in rabbits regularly produced glycosuria. In dogs his results were inconclusive. Out of 7 animals 2 failed to give sugar in the urine, and in the other cases extracts of 20 to 30 glands were injected. The blood of 2 rabbits showed hyperglycaemia. The glycosuria was transitory, coming on three hours after the injections and rarely lasted through the succeeding twenty-four hours. Rossi² reports glycosuria in dogs following the injections of hypophyseal extracts.

Franchinini³ attempted to repeat Borchardt's results using the latter's technique for the preparation of the extracts. For his experiments he used 22 rabbits, and in only 2 of these was he able to produce glycosuria, which appeared towards the end of life. Autopsies on these animals showed ulcerations and haemorrhages in the duodenum and the small intestines. In his criticism of the positive findings of Rossi, he calls attention to Rossi's use of 0.5 per cent phenol for a preservative, a substance which has been shown by Bukowski⁴ to cause glycosuria. He is inclined to believe that there is no specific action in the extracts, but whatever glycosuria may result is attributable to the lesions in the intestinal tract. In support of this view he cites two cases⁵ of similar lesions in man, produced by swallowing aqua regia and sodium hydroxide, attended by a glycosuria preceding death. Franchinini cites a

¹ Borchardt, L.: *Zeitschr. f. Klin. Med.*, 1908, lxvi, 332.

² Rossi: quoted by Franchinini, *Loc. cit.* Il Tommasi, 1909, No. 25, 26. 592.

³ Franchinini: *G. Berl. Klin. Wochenschr.*, 1910, xlvii, 670.

⁴ Quoted in Borchardt's article.

⁵ Zak, E.: *Wiener Klin. Wochenschr.*, 1908, No. 3, p. 82.

short note by Pal,⁶ who agrees with him in getting negative results with hypophyseal extracts.

Goetsch, Cushing, and Jacobsen⁷ were able to confirm Borchardt as to the production of glycosuria in rabbits. They also found that injections of posterior lobe extract lowered the tolerance of dogs for cane sugar, but no data is offered on the production of glycosuria in these animals on a normal mixed diet. Following surgical manipulations which involved the crushing of the stalk, as in total hypophysectomy, a transient glycosuria resulted; if however a clean-cut posterior lobe enucleation was performed no sugar appeared. This latter result was one which grew out of a series of hypophysectomies by Crowe, Cushing, and Homans,⁸ and appeared to be so constant in their experience that no specific data is presented. In studying the presence of the infundibular lobe secretion in the cerebro-spinal fluid Cushing and Goetsch⁹ placed a silver clip on the stalk of the gland in three cases. Two of these showed glycosuria.

Weed, Cushing, and Jacobsen¹⁰ in pursuance of their studies into the control of the hypophysis in carbohydrate metabolism, attacked the problem by hypophyseal puncture, by stimulation of the gland and the superior cervical ganglion electrically. The hypophyseal puncture in rabbits gave glycosuria, a result also obtained by a typical Bernard puncture, even after section of the cord at the level of the fourth thoracic vertebra. In the latter case it was found necessary to allow a sufficient time for the re-accumulation of hepatic glycogen. Stimulation of the superior cervical ganglion (rabbits) and of the gland (cats), after transection of the cord and the reaccumulation of glycogen caused "a prompt and outspoken glycosuria." These results fail if the posterior lobe has been removed from the animal. Rabens and Lifschitz¹¹ repeated the stimulation of the superior cervical ganglion without an anaesthetic, and estimated the reducing power of the blood, with entirely negative results. They called attention to the lack of published controls of Cushing and associates, and suggested that their results might be attributed to the anaesthesia.

⁶ Pal: *Semaine Medicale*, 1909, xxvi, 312.

⁷ Goetsch, E., Cushing, H., and Jacobsen, C.: *Johns Hopkins Hospital Bull.*, 1911, xxii, 165.

⁸ Crowe, S. J., Cushing, H., and Homans, J.: *Ibid.*, 1910, xxi, 127.

⁹ Cushing, H., and Goetsch, E.: *This Journal*, 1910, xxvii, 60.

¹⁰ Weed, L. H., Cushing, H., and Jacobsen, C.: *Johns Hopkins Hospital Bull.*, 1913, xxiv, 40.

¹¹ Rabens, I., and Lifschitz, J.: *This Journal*, 1915, xxxvi, 47.

As to the cause of the glycosuria, Goetsch, Cushing, and Jacobsen state "We assume that the spontaneous glycosuria represents a hyperglycaemia from the discharge of stored glycogen, which has been set free by the introduction into the circulation of the posterior lobe secretion." Following this and in the work of Weed, Cushing, and Jacobsen, the reader gains the impression that such an assumption has been fully established and adequately proven. The lowering of the tolerance of dogs for cane sugar by injections of posterior lobe extracts, the necessity of "available glycogen" for the production of glycosuria on stimulating the gland, and the two cases of hyperglycaemia in rabbits (Borchardt) renders highly probable indeed the assumption, but it does not constitute final proof.

It seemed to us highly important to extend if possible the results of Weed, Cushing, and Jacobsen on stimulation of the gland to dogs, since their stores of glycogen are not so mobile as those of the cat and rabbit. We felt that by estimating the reducing power of the blood we had a more delicate method of following the effects of stimulation of the gland, and a means of determining finally the cause of the glycosuria, whether it is due to a true hyperglycaemia, or a lowered threshold for sugar presented by the animal under this experimental condition.

B. EXPERIMENTAL METHODS

Anaesthetic

Any experimental study of the sugar in the blood or urine, which involves the use of an anaesthetic demands the most rigorous set of controls. Macleod¹² found that the stimulation of the central end of the vagus nerve of the dog and rabbit under ether anaesthesia led to hyperglycaemia and glycosuria. If these same operations were repeated with oxygen insufflation no change in the sugar level resulted. In his discussion of these results he has to say,

Although at first sight these results would seem to indicate that the vagus cannot carry afferent fibers to the glycogenic center; this conclusion is not inevitable for it is possible that with the over-arterialization of the blood a small increase in the amount of sugar delivered into it would not cause hyperglycaemia, because of the excess of sugar being burnt up.

He has further noted that oxygen insufflation diminishes the hyperglycaemia following the stimulation of the splanchnic nerve, but it

¹² Macleod, J. J. R.: Diabetes. Longmans and Co., 1913, p. 61, 79.

does not abolish it entirely. Upon this basis he has opposed the use of an anaesthesia, which involves positive ventilation of the lungs, wherever it could be avoided.

More recently Shaffer and Hubbard¹³ using the artificial respiration apparatus designed by Gesell and Erlanger¹⁴ have studied the reducing power of the blood, and find that remarkably low levels can be maintained during operative procedure lasting even for several hours. They strongly urge the advantage of working at these lower levels, and suggest that forced respiration be established in all cases, where variations of the blood sugar are being observed. It seemed necessary to adopt some method of ventilating the lungs in the administration of the anaesthetic, since stimulation was to be applied to different points of the brain, a procedure most likely at some time to result in the alteration of the respiratory rhythm. Our method consisted in insufflation with a small continuous air current, led through a tube terminating in the lower cervical trachea. By adjusting the stream carefully the normal spontaneous respiratory rhythm of the animal could be maintained. The cervical location of the tube avoids the possible depressor effects on blood pressure that might supervene in a thoracic location, as shown by Janeway and Ewing.¹⁵

There is a variable rise in the reducing power of the blood in putting an animal under ether anaesthesia. Our experience has led us to the conclusion, that of the two factors involved, asphyxia, due to the methods of administration of the ether, holding of the breath of the animal, and excitement, the former is much the more important. With an open towel administration of the anaesthetic, attention to the tongue and mucous in the throat, the experiment can be started with a level of 0.10 per cent to 0.13 per cent reducing substances figured to dextrose. Faulty manipulation can just as readily result in beginning with a 0.18 per cent to 0.2 per cent level. Preliminary experimentation with our method of anaesthesia soon showed us that the reducing substances in the blood did not increase with two to three hours of ether administration, but as a rule fell. It is not necessary to quote extensively data from these experiments, since the large number of cases unattended by a rise cited in the body of the paper establish the method as satisfactory. However two experiments with strychnine, which

¹³ Shaffer, P. A., and Hubbard, R. S.: *Proc. Amer. Soc. Biol. Chem.*, 1914, iii, 31.

¹⁴ Gesell, R. A., and Erlanger, J.: *This Journal*, 1914, xxxiii, p. xxxiii.

¹⁵ Janeway, H. T., and Ewing, E.: *Ann. of Surgery*, Feb., 1914, lix, p. 159.

was administered in quantities sufficient to cause marked respiratory embarrassment, demonstrate the readiness with which an asphyxial hyperglycaemia may arise and the efficacy of our method in preventing it.

Etherization with tracheal cannula

The animal was kept under ether for three hours, during which time 8 mgm. of strychnine sulphate in 2 mgm. doses was given intravenously. By lightening up on the ether, and stimulating the animal with a series of taps on the nose, he was kept in distinct tremors most of the time. The reducing power of the blood increased from 0.200 per cent to 0.377 per cent.

Etherization with insufflation tube

Animal was under ether two hours, 4 mgm. of strychnine sulphate was given. Tremors were maintained throughout the period. Reducing power of the blood showed no change, being 0.101 per cent at the beginning and 0.098 per cent at the close.

Operative procedure

The buccal method of exposing the gland was adopted, because it demands the minimum of operative interference, and gives a satisfactory field for stimulation. The use of a dental drill and wax makes a complete exposure of the gland possible in a short time, even in the face of persistent haemorrhage from the bone. The electrodes were plunged through the dura into the gland and stimulation with a tetanizing current applied for twenty to thirty minutes. If the dura is not removed, the chances of recovering the animal for subsequent stimulations are good although no aseptic precautions are taken.

We have studied the stimulation of the gland also by burying iron filings in it, and then exposing them to an electromagnet. Such a method enables us to dispense with an anaesthetic. Our results will form the substance of a later report.

Estimation of the reducing power of the blood

The blood (6 cc. samples) was drawn from the jugular vein into a weighed beaker containing 10 per cent anhydrous sodium sulphate in 1.5 per cent acetic acid, which was immediately reweighed. The beaker was then immersed in a calcium chloride bath at 117° for six minutes, contents filtered, and washed with a definite quantity of the

acid sulphate solution. The reducing power of the filtrate was then estimated by the Bertrand method, the titration being conducted with a permanganate solution previously standardized against a known dextrose solution in the same salt concentration. One cc. of the permanganate was equal to 1 mgm. of dextrose. Occasionally when difficulty was experienced in drawing the samples because of clotting, or when for any reason the washing was not done in the same empirical fashion, the duplicates did not check as closely as one desired. However in no case were there variations sufficient to invalidate the conclusions. We feel that the withdrawal of a sample of sufficient size to give duplicate estimations on aliquot parts would eliminate these variations, but it would also involve the removal of 25 per cent more blood, a factor not to be overlooked.

C. RESULTS

1. The effect of drilling to expose the gland

It was early evident that the mere act of drilling over the gland in the course of its exposure caused a rise in the reducing power of the blood. With the idea of studying this factor and with the hope of stimulating the gland in the absence of the preliminary rise in the reducing power of the blood, it was decided to remove the bone from a number of animals, allow them to recover, and stimulate the gland at some subsequent date. In the first four the whole roof of the mouth from the optic chiasma to well behind the gland was removed. In five others three holes (one over the gland, and the others in front and behind it), separated by bony bridges were drilled in position. A summary of the results follows in Table I.

It thus appears that where the manipulative procedure was extensive ("Exposure of the whole roof of the mouth"), there was a rise in the reducing power of the blood; but where it was not so extensive ("Three holes"), the rise did not always follow (5, 6, 7).

2. The effect of stimulation of the gland

A glance at Table II shows the unmistakable rise in the reducing power of the blood following stimulation of the gland, and that this rise is not dependent upon the drilling is evidenced by dogs 3 and 15.

In only one case (dog 9) was there no rise in the reducing power of the blood. This animal was suffering from the distemper, and had

eaten little or nothing during the previous week. In dog 6 the rise is not so marked as in the other cases. This result may be attributed to the annoying oozing of fluids into the field so that it was impossible to get a clean-cut stimulation of the gland.

3. The effects of stimulation anterior to the gland

An analysis of Table III, which summarizes the results of stimulation anterior to the gland shows that in 3 of the dogs (2, 7, and 17)

TABLE I
Effect of drilling on the reducing power of the blood

ANIMAL	REDUCING POWER OF THE BLOOD EXPRESSED IN PER CENT OF GLUCOSE		
	Before calling	After drilling	20-30 min. rest
<i>Exposure of the whole roof of the mouth</i>			
1.....	0.086	0.121	
	0.088	0.115	
2.....	0.113	0.109	0.160
	0.117	0.108	0.170
3.....	0.161	0.185	
	0.157	0.187	
4.....	0.168	0.190	0.192
	0.155		
	Three holes		
5.....	0.042	0.050	0.050
	0.052	0.049	0.041
6.....	0.110	0.143	0.134
	0.135	0.146	0.143
7.....	0.062	0.067	0.076
	0.069	0.069	0.073
8.....	0.190	0.210	0.233
	0.199	0.204	0.229
9.....	0.084	0.150	0.160
	0.090	0.160	0.164

there was no rise in the reducing power of the blood, in the others (16, 18, 19) there was a rise of a much lower grade than that found in the cases where the gland was stimulated. Autopsies of the animals with the electrodes in position showed their locations from 0.5 to 3 mm. anterior to the hypophysis.

TABLE II

Effect of stimulation of hypophysis with tetanizing current

ANIMAL	REDUCING POWER OF THE BLOOD EXPRESSED IN PERCENT OF GLUCOSE			
	Before drilling	After drilling	Stimulation 20-30 min.	Rest of 20-30 min.
2.....	0.175	0.211	0.237	0.247
	0.183	0.206	0.238	0.245
3.....	0.072	*	0.196	0.198
	0.082	*	0.196	0.197
6.....	0.115	0.139	0.171	0.166
	0.119	0.130	0.165	0.169
9.....	0.053	*	0.055	0.042
	0.044	*	0.048	0.039
10.....	0.216	0.297	0.488	0.313
11.....	0.160	0.210	0.180	0.210
12.....	0.096	0.132	0.160	0.160
13.....	0.155	0.193	0.226	0.217
	0.165	0.203	0.230	0.217
14.....	0.176	0.202	0.206	0.214
	0.172	0.198		0.213
15.....	0.133	*	0.216	0.255
	0.140	*	0.206	0.251

* Previously drilled out.

TABLE III

Effect of stimulation anterior to the hypophysis

ANIMAL	REDUCING POWER OF THE BLOOD EXPRESSED IN PERCENT OF GLUCOSE			
	Before drilling	After drilling	Stimulation 20-30 min.	Rest of 20-30 min.
2.....	0.189	*	0.171	0.176
	0.200		0.165	0.182
7.....	0.053	*	0.065	0.066
	0.061		0.067	0.067
16.....	0.139	0.143	0.177	0.161
17.....	0.062	0.062	0.085	0.080
	0.054	0.060	0.098	0.077
18.....	0.046	0.037	0.078	
	0.035	0.030	0.073	
19.....	0.111	0.149	0.169	0.170
	0.094	0.148		0.182

* Previously drilled out.

4. *The effect of stimulation posterior to the gland*

In only one case of Table IV (dog 20) was there a significant rise in the reducing power of the blood on stimulation applied at a point posterior to the gland. The technique of experiments 2, 5, 6, and 9 was all that could be desired, for the field had been previously uncovered and the gland was insulated from escaping currents with dental wax. These results and those obtained from the stimulations anterior to the gland show in our estimation that it is possible to stimulate the floor

TABLE IV
Effect of stimulation posterior to the hypophysis

ANIMAL	REDUCING POWER OF THE BLOOD EXPRESSED IN PERCENT OF GLUCOSE			
	Before drilling	After drilling	Stimulation 20-30 min.	Rest of 20-30 min.
2.....	0.193	*	0.167	0.203
	0.201		0.167	0.190
5.....	0.072	*	0.067	0.061
	0.066		0.061	0.060
6.....	0.110	*	0.125	0.137
	0.102		0.128	0.137
9.....	0.096	*	0.061	0.070
	0.089		0.067	0.071
20.....	0.250	0.255	0.280	
	0.246		0.295	
21.....	0.100	0.107	0.095	
	0.085	0.095	0.103	

*Previously drilled out.

of the brain in the vicinity of the hypophysis without increasing the reducing power of the blood.

Autopsies confirmed the locations of the stimulations as from 2 to 4 mm. posterior to the gland.

5. *Stimulation of the gland in animals whose splanchnic nerves have been previously cut*

A table (V) is appended summarizing the results of the stimulations of the gland in animals whose splanchnic nerves had been previously sectioned. Attention is called to the fact that with one exception (22) the nerves had been cut from thirty to one hundred and thirty-seven

days before the experiment. These animals with the exception of 26 had been confined within the cages and had not been allowed the run of the paddock, hence they should have had a supply of "available glycogen" (Weed, Cushing, and Jacobsen). Dog 27 had in addition a double vagotomy, below the heart and above the diaphragm, performed five months previous, so we feel certain that his liver was free of all connection with the central nervous system. The only suggestion of a rise is found in cases 22 and 24, but this is not at all comparable to that found in normal hypophyseal stimulation.

TABLE V

Effect of stimulation of the hypophysis in dogs whose splanchnic nerves have been previously sectioned

ANIMAL	REDUCING POWER OF THE BLOOD EXPRESSED IN PER CENT OF GLUCOSE				
	Before drilling	After drilling	Stimulation 20 min.	Rest 20 min.	Days after section of splanchnics
22.....	0.107	0.125	0.120 0.130	0.125 0.125	10
23.....	0.074 0.075	0.059 0.062	0.059 0.052	0.055 0.055	75
24.....	0.114 0.114	0.116 0.109	0.131 0.132	0.129 0.137	60
25.....	0.115 0.105	0.064 0.075	0.071 0.078		90
26.....	0.088 0.098	0.077 0.087	0.031 0.039	0.053 0.043	30
26.....	0.078 0.073	* *	0.062	0.058 0.065	70
26.....	0.102 0.101	* *	0.049 0.041	0.045 0.039	137
27.....	0.056 0.051	0.056 0.054	0.051 0.050	0.056 0.064	108

*Previously drilled out.

6. Glycosuria in relation to the reducing power of the blood

Having in mind the diuretic effects of gland extracts, we felt that the glycosuria might be partially attributable to the lowering of the threshold of the kidney for sugar, a phloridzin effect, so simultaneous estimations were made on the reducing power of the urine and blood in thirteen cases. Of seven cases so studied, which did not present glycosuria, the maximum values of reducing power of the blood were 0.110,

0.110, 0.133, 148, 160, and 177 per cent. In those cases presenting sugar, the lowest levels were 0.191 and 0.21 per cent. These values agree well with the threshold as determined by Pollak¹⁶ for rabbits under conditions of diuresis. For a diuretic 10 per cent sodium sulphate was used in 5 to 10 cc. doses depending on the size of the animal.

After the establishment of glycosuria, the sugar increased rather rapidly as the result of stimulation of the gland, and was continued into the succeeding rest period. As an illustration a protocol of dog 13 may be cited.

Dog 13

After anaesthesia, blood reducing power, 0.159 per cent; urine, 0.87 per cent.

After drilling, blood reducing power, 0.190 per cent; urine, 3.96 per cent.

After stimulation, blood reducing power, 0.228 per cent; urine, 5.12 per cent.

After rest of 20 minutes, blood reducing power 0.217 per cent; urine 5.78 per cent.

Macleod¹⁷ in studying the behavior of glycosuria resulting from stimulation of the splanchnic nerve observed that, having once established itself, the sugar in the urine increased out of proportion to that in the blood, and that its return to a normal occurs at a later period. These results suggest a toxic action exerted by the sugar on the kidney cells. Or we may be dealing with a question of habit formation as shown by Mostrom and McGuigan¹⁸ for strychnine convulsions in frogs. Once the kidney cells have responded to a given level of sugar in the blood, they appear to continually lower their threshold with increasingly larger quantities of sugar in the urine. Indeed the clinical therapeutic measure of establishing higher degrees of tolerance in diabetic cases points in the same direction. While we have not followed out the return to normal in hypophyseal glycosuria, and while we feel that our data is too meager to be conclusive, we wish to point out the apparent analogy of this glycosuria to that resulting from splanchnic stimulation.

D. DISCUSSION AND CONCLUSIONS

We feel that the evidence presented in the foregoing section establishes that it is possible to stimulate in the vicinity of the hypophysis without causing a rise in the reducing power of the blood. That stimulation

¹⁶ Pollak, L.: Arch. f. Exp. Path. u. Pharm., 1909, lxi, 157.

¹⁷ Macleod, J. J. R.: Loc. cit., p. 46.

¹⁸ Mostrom, H. T., and McGuigan, H.: Journ. Pharm. and Exper. Therap., 1912, iii, 515.

of the gland causes a rise in the reducing power of the blood in dogs may be regarded as settled. We believe further that any stimulation in this region must be effective only in proportion as it throws the gland into activity. Of course our evidence on this point is not logically complete, for we have not examined every point outside of the hypophysis, nor were all of our experiments on locations anterior to the gland negative. However it was not always possible to produce technically perfect experiments on account of oozing fluids, which must have served as conductors of the currents. The really important question is not whether 50 per cent of our experiments were positive or negative, but whether we were able as the result of improved technique to get a sufficient number of undoubted stimulations in the neighborhood of the hypophysis, which did not cause a rise in the reducing power of the blood. Our protocols establish this clearly.

The drilling constitutes a mechanical stimulus to the gland, and the rise in the reducing power of the blood is explained on this basis. The height attained is less than under electrical stimulation, hence it is not so efficient a stimulus. If the drilling is done carefully, quickly, and with no trauma to the hypophysis, the rise may be absent.

Our results with dogs, whose splanchnic nerves have been cut, are diametrically opposed to those of Weed, Cushing, and Jacobsen on cats and rabbits with cord sectioned at the fourth thoracic vertebra. They are of the opinion that the stimulation of the gland is responsible for the liberation of an internal secretion, which produces glycosuria through a hyperglycogenolysis, since all nervous connections to the liver have been severed. A glance at many of their protocols shows that the stimulations followed within two or three days of the section of the cord. In one instance (XXVIII) although the urine of the animal showed a positive Fehling's at 8.30 a.m., yet a negative one at 12.45 p.m. was deemed a sufficient precaution to allow of etherization and stimulation of the gland at 1.30 p.m. The question arises whether the instability of the blood pressure regulating mechanism has been carefully enough considered. In the absence of proof to the contrary two or three days' time appears a rather short interval to allow for recovery of vaso-motor tone. On this point Sherrington¹⁹ has to say,

When in the dog complete transection of the spinal cord through the eighth cervical segment is practiced, a severe fall in the general arterial pressure ensues, and vasomotor reflexes cannot be elicited. But in the course of *some* days this is

¹⁹ Sherrington, C. S.: Integrative action of the nervous system, 1911, p. 241.

largely recovered from, and after *some* weeks the blood pressure will, with the animal in the horizontal position, often be found practically normal.

A dog with splanchnics cut one to two months previous and in good health seems to us a more reliable experimental animal than a cat or rabbit with a sectioned cord. Hill²⁰ states that tone is completely restored to the splanchnic area some eight days after section of all of the splanchnic nerves.

While our results on their face appear to speak against the liberation of a hormone causing the hyperglycaemia, yet this does not necessarily follow. Such a hormone might find its site of action on the terminations of the splanchnic nerves or on the neuro-cellular junction, either in the adrenals or in the liver. If this is the solution of the case, then sectioning of the cord as practiced by Weed, Cushing, and Jacobsen ought to have no effect on these terminations; while our division of the splanchnics would in all probability destroy the fibers to the adrenals, but might not interfere with the activity of those to the liver. For in the case of the adrenal the medulla originates from the same blastema as the peripheral sympathetic ganglia, and thus might be looked upon as the homologue of the postganglionic fiber. On this basis our cut would have destroyed the one neuron to the adrenal. The fibers to the liver relay in the great plexus intimately associated with the branches of the aorta, so our section has destroyed only the pre-ganglionic fibers, leaving the postganglionic ones intact. It has been further suggested to us²¹ that the site of action of the hormone might be some central glycogenic center, which required an intact nervous pathway to the viscera for its discharges. We are justified however in concluding that our experiments speak against a hormone, which affects the liver and muscle cells directly causing an increased glycogenolysis. In contrast to the hormone regulation the possibility of a more or less direct nervous route from the hypophysis to the glycogen stores offers itself as an obvious mechanism which will have to be investigated.

CONCLUSIONS

Our results may be summarized as follows.

(1) If precautions are taken to avoid asphyxia, an animal may be anaesthetized with ether without causing a marked rise in the reducing power of the blood. Such a level once established not only does not tend

²⁰ Hill, L.: Shaeffer's textbook of physiol., 1900, ii, 138.

²¹ Dr. A. J. Carlson, University of Chicago.

to increase but generally falls under one to three hours of insufflation anaesthesia.

(2) Electrical stimulation of the hypophysis in dogs under insufflation anaesthesia gives rise to an increase in the reducing substances in the blood. Drilling over the sella stimulates the gland mechanically, but not so efficiently as the induced shocks.

(3) If the stimulation is applied anteriorly or posteriorly to the gland, with precautions to prevent an escape of the current to the hypophysis, no rise in the reducing substances results.

(4) This rise on stimulating the gland does not occur in dogs whose splanchnic nerves have been previously sectioned, a fact which argues against the liberation of a hormone, which increases directly the cellular glycogenolysis.

(5) With active diuresis the threshold of glycosuria lies between 0.190 per cent and 0.21 per cent reducing power of the blood figured to dextrose. Once established the sugar in the urine increases in concentration out of proportion to the reducing power of the blood.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH
XXIX. THE GASTRIC HUNGER CONTRACTIONS OF THE NORMAL AND
DECEREBRATE GUINEA-PIG

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INTRODUCTION

Carlson (1) has shown that in the dog and man gastric hunger movements may be modified by psychic stimuli, though only in the direction of inhibition, and that in the dog they continue even when the stomach has been isolated from the central nervous system by section of the vagi and splanchnic nerves. After such section inhibition of tonus and contractions may still be obtained by stimulation of the gastric mucosa, but it is diminished both in intensity and duration. Cutting of the vagi leaves the stomach in a permanently hypotonic condition (2). Without doubt the central nervous system acts merely as one of the regulators of this reflex mechanism but for how much of the regulation the cerebrum is responsible and what part is played by the midbrain and medulla oblongata, can be determined only by observing a decerebrate animal.

Hoping to throw some light on the question, a study of the gastric contractions in normal guinea-pigs was undertaken and subsequently a study of such contractions after removal of the cerebrums. It is well known that this animal survives decerebration for several hours (3) and special reference should be made to the observations of Brown after unilateral (4) and complete (5) removal of the cortex. Sherrington (6) includes the guinea-pig among the animals in which the symptoms of decerebrate rigidity occur with little variation.

PRESENT INVESTIGATION

For observations on the stomach contractions gastric fistulas were made in our animals and the recording method described by Carlson (7) was employed. Sixteen animals weighing from 450 to 790 grams were studied over periods varying from 13 to 66 days. The guinea-pig is so foreshortened that the operation was beset with some difficulties—the fundic portion of the stomach is pushed up under the diaphragm in such a manner that it must be pulled downward and stitched to the abdominal wall to make a fistula, or an opening must be in the pyloric region. Both methods proved satisfactory and the possible objection to the lower opening that the balloon did not lie in the fundus was obviated by the use of a small balloon or of a finger cot from 3 to 5 cc.

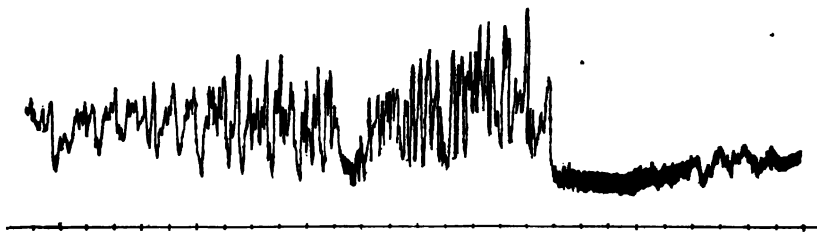


Fig. 1. Tracing showing the termination of a period of gastric hunger contractions of a guinea-pig, five hours after feeding. Note the gradually increasing intensity of the contractions and the incomplete tetanus which ends the period. Three hours later, when the balloon was removed, the stomach contained food. Time: 27 minutes.

This and the following figures were retraced from the original tracings by the author.

in capacity, pushed well up into the stomach. Several animals killed with the balloon in place, left no doubt of the ease with which it was properly inserted. Very small fistulas were made so that when the wounds healed they often measured less than a centimeter across. Since the animals began eating within twelve hours, the food in the distended stomach prevented the openings from closing. A dressing was unnecessary after the fourth or fifth day but vaseline was applied daily during the observation period. In recording a water manometer was used, the pressure varying between 3 and 4 cms.

The guinea-pig, like other herbivorous animals, feeds at frequent intervals—probably every hour—and under normal conditions the stomach is never found empty. Even within two hours after exclusion

from food it begins eating its own excreta, a fact already reported (8), and after twelve hours will eat paper, pasteboard or anything of that nature within reach. The easiest and most effective method found for excluding it from its own feces was to place all but the head in a bag, sufficiently small to prevent much freedom of movement, and then to draw the bag closely about the neck.

Rogers (9) has reported the appearance of gastric hunger contractions in the rabbit twelve hours after its exclusion from food and excreta. In the guinea-pig a similar type of contraction was recorded in five hours (fig. 1). Frequently continuous records were made from the time the food was removed until the onset of such vigorous movements. The mild peristaltic waves become more and more intense until contractions such as might be classified as type I (2) appear—periods of feeble tonus lasting two or three minutes with four or five superimposed contractions. This type may continue for four hours but, as in figure 1, they gradually merge into the more vigorous type II and possibly III. The contractions follow one another in rapid succession—one in eighteen seconds (average)—such a period terminating in complete quiescence of the stomach. At times a period of violent coughing precedes the inhibition. Contractions of types I and II have been recorded continuously for six hours with but two periods of rest lasting eight and six minutes respectively. In figure 1 the short period of inhibition was followed by contractions of gradually increasing intensity which continued for three hours when the record was discontinued on account of the animal's restlessness.

The subject's voluntary movements—turning about and licking itself—may cause the termination of a hunger period. Sudden noises or talking close by usually have an inhibiting effect though records started amid the noise of a general laboratory were sometimes as satisfactory as those secured with quiet surroundings. Substances which cause inhibition when placed in the stomach of man or the dog or when they stimulate the end-organs of taste, are without effect on the guinea-pig. Water was introduced, 0.5 per cent hydrochloric acid, sugar solution, and quinine by a pipette into the mouth, some was always swallowed though probably scarcely half a cubic centimeter. All these substances, like the acid (fig. 2, *a*) were negative in their effect, whether the contractions were mild or intense. It was difficult to introduce substances directly into the stomach without attracting the animal's attention, for the presence of an extra tube passing through the fistula usually proved irritating and prevented the onset of hunger

movements. In a few instances, however, we succeeded in introducing about 1 cc. of the various substances but with negative results. Smelling and tasting food—when the animal remained quiet—produced no inhibition but we were surprised to find that even the chewing and swallowing of the most appetizing substances such as clover or lettuce also gave negative results (fig. 2, b). To be sure a hungry animal frequently became so restless as soon as food was given that further observations were impossible but such a record as the one shown, taken during the eleventh hour of fasting, has been repeatedly obtained. Vigorous contractions like those at either end of this tracing had gone on for nearly four hours without inhibition.

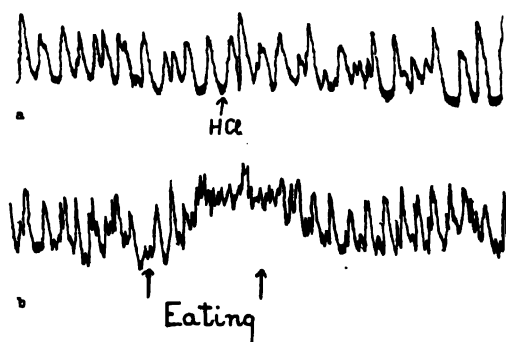


Fig. 2. Tracing showing hunger contractions of guinea-pigs—*a*. Failure of 0.5 per cent HCl, given by mouth to cause inhibition. Time: 11.5 minutes. *b*. Failure of chewing and swallowing food to cause inhibition. Time: 16.5 minutes. In both instances the stomach contained food.

tions on the difficulty of inhibiting the hunger movements are in agreement with those reported for the rabbit (9), are indeed even more pronounced. An animal fasted for such periods as twenty-four and forty-eight, hours was exceedingly restless and long continued records were not possible, but the periods of increased tonus or tetany are more frequent than during the earlier hours.

An interesting peculiarity and one of some significance, is the periods of inhibition often noticed in the midst of normal digestion peristalsis (fig. 3). They are of about the same duration as those terminating a hunger period—eight to ten minutes—and come on when there are no outside disturbing factors. Their occurrence emphasizes the close

guinea-pig quietly but eagerly ate lettuce. The movements continued for ten minutes then it was allowed to eat for five minutes, the same type of contraction was recorded for fifteen minutes, following which the subject became very restless and the observations were discontinued. In other instances more food was given and the onset was watched of normal digestive peristalsis, occurring after about half an hour. These observa-

relation existing between the movements of the stomach during digestion and those during hunger. Rogers' (9) observation for the rabbit that "gastric hunger contractions are intensified peristalsis" may undoubtedly be made for the guinea-pig as well.

That discomfort is experienced when food is withheld for even four or five hours is evidenced by restlessness, the eating of the animal's own excreta, chewing movements and sometimes crying when the contractions are unusually vigorous. But why such hunger should be experienced while the stomach still contains abundance of food (it not only adheres to the balloon when removed but comes from the fistula) is difficult of explanation. The temperature of the normal guinea-pig taken by fistula is 103.2° F., perhaps the active metabolism of the animal, due to its small size and almost constant activity, explains its constant need of food.

EFFECT OF DECEREBRATION ON THE HUNGER CONTRACTIONS

Since psychic inhibition plays such an unimportant rôle in the control of the hunger mechanism of the guinea-pig, we did not expect to find the stomach

movements
modified by re-
moval of the
cerebrum—when
time had been
allowed for re-



Fig. 3. Tracing showing the inhibiting of normal digestion peristalsis in the undisturbed guinea-pig. Compare this with the similar period in figure 1. Time: 13 minutes.

covery from the shock of the operation. Ten animals were decerebrated, none lived less than eighteen hours, some nearly thirty-six and one was killed at the end of seventy-five hours. The lesions were made by a thermocautery so that excessive hemorrhage might be prevented. Later the lesions made were located by sectioning the brains and mounting every tenth section, or by examination of the gross sections. The cortical lesion, in nearly every instance, was so extensive as to leave no doubt that its functional significance was destroyed. The corpora striata were penetrated, in some cases much more extensively than in others, and in a few the lesion included a portion of the optic thalami.

A rather detailed description of the symptoms of one animal may be taken as typical.

The guinea-pig was taken from its food and quickly decerebrated.

By the time the wound was closed it was able to walk about and exhibited the usual symptoms—repeated shaking of the head, restlessness, struggling when restrained and very rapid rate for heart and respiration (these could not be counted). A little later other observations were made—it would start violently when the skin was pinched or the leg pulled, would fall from the table when allowed to wander freely about, or would beat its head against the sides when placed in the cage. No notice was taken of objects passed before its eyes unless the lashes were touched, if the hands were clapped close by, the ears were moved as in the normal animals. It struggled and cried when the balloon was inserted—normal animals offer very little resistance. During the first day, periods of quiet were broken by extreme restlessness—turning about, licking itself, biting at the edge of the box or at the fingers of the investigator. After twenty-four hours the animal was less active and would sit for an hour at a time with neck drawn in and hair roughed up, resembling the attitude of a decerebrate pigeon. On the third day it was very weak and lay on its side much of the time while records were being taken, its limbs were rigidly extended and trembling. During the night succeeding it was observed at frequent intervals, being more active than during the day, the animal frequently climbed from its box and would be found lying on its back or side struggling aimlessly, or suspended on the edge unable to get in or out. On the fourth day it lay on its side much of the time and was killed at the end of seventy-five hours. During the period of observation water was frequently given by the fistula, a little could be swallowed if placed far back on the tongue by a pipette. An examination of the brain showed complete destruction of the cortex on the dorsal and lateral surfaces, the cortex of the hippocampal lobes and the olfactory lobes was not injured. The lesion penetrated the corpora striata and the thalami.

Some of the animals were much more active than the one described and were able to jump from a box a foot deep. Most of them exhibited spasmodically the symptoms of decerebrate rigidity—limbs extended and head drawn backward. In all cases records were obtained in spite of the excessive activity. In six there were periods of quiet continuing for half an hour or more. Gastric movements were recorded, in the guinea-pig described above, six hours after the operation and figure 4 (a), the close of the seventh hour, shows the type of contraction. The subject was remarkably quiet and the inhibition which terminated the hunger period had lasted for four and a half minutes, then mild peristaltic contractions set in which merged into those of type I. These

continued for half an hour when the animal became so active that the inflated balloon was pulled from the stomach. Abundance of food adhered to it and came from the fistula. The marked degree of tonus shown in these contractions characterized those of the decerebrate guinea-pig. The tonus periods (fig. 4, *a*) lasted from a minute to one and a half and a superimposed contraction appeared every ten seconds—twice as frequently as in the normal. In this animal the tonus was less pronounced after the first day (fig. 4, *b*), although the contractions occurred at the same rapid rate, but others showed it in unusual degree for twenty-four and thirty hours. The tonus continued but the gastric movements gradually became less vigorous until on the fourth day when only respiratory movements and very feeble contractions were recorded. Water, etc., given by the mouth caused no inhibition, no attempt was made to introduce it directly into the stomach while recording.

The striking variations, then, of the decerebrate from the normal animal are—an increased tonus of the musculature of the stomach and an increase in the rate of contraction. For the

normal these average two to three per minute as opposed to six in the operated animal. Periods of inhibition were usually briefer than in the normal but as to the frequency of their occurrence an accurate statement is impossible, for hunger contractions were so often terminated by the animal's activity. The restlessness of the decerebrate guinea-pig as of Goltz's dog, may be attributed to the absence of inhibitory impulses from the cerebral cortex. In the normal animal such impulses must be continually influencing the gastric movements—decreasing their rate and lowering the muscular tone. Since section of the vagi in the dog (2) leaves the stomach in a hypotonic state and removal of the cortex, in the guinea-pig at least, is followed by

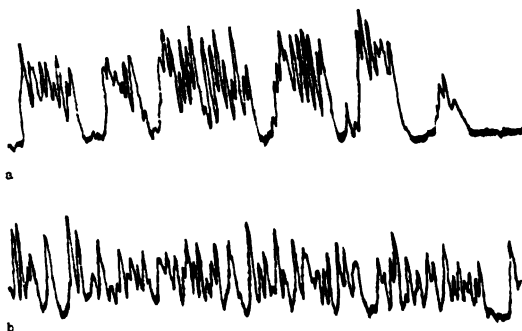


Fig. 4. Tracings showing contractions obtained from a decerebrate guinea-pig: *a*. Termination of a hunger period 7 hours after the operation, 7.5 hours after feeding. Note the marked periods of tonus. Stomach contained food. Time: 11 minutes. *b*. Hunger contraction from same animal, 25 hours after operation. Stomach empty.

a condition of hypertonus we may infer that impulses from centers in the mid-brain and medulla and not those from the cerebrum exercise the controlling influence.

SUMMARY AND CONCLUSIONS

1. Hunger contractions, comparable to those observed in other animals, appear in the guinea-pig from four to five hours after feeding, while the stomach is still well filled.

2. Water, 0.5 per cent hydrochloric acid and other substances which usually cause inhibition of such contractions give negative results, whether swallowed or placed directly in the stomach. After eating, the vigorous movements continue for about half an hour and then merge into those of the mild peristaltic type.

3. The normal peristaltic movements of digestion may be interrupted by intervals of quiescence such as terminate a hunger period. This fact adds additional weight to the suggestion of Carlson that hunger contractions are simply more vigorous peristaltic movements.

4. After decerebration, contractions of a similar character are recorded but showing a marked increase in rate, the stomach being in a hypertonic condition. It is held that the absence of inhibitory impulses from the cerebral cortex accounts for these striking variations, the positive influence of the brain on stomach motility originating below the cerebrum.

The problem of this study was suggested by Professor Carlson and his kindly interest and criticisms of the work are gratefully acknowledged.

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1915

NEURASTHENIC WOMEN

"Certainly our most striking results have been obtained with a class of patients usually described as neurasthenics. Most of them are over 35 years of age."—Curtis F. Burnam, M. D., "The Journal," A. M. A., August 31st, 1912, p. 698.

"THE STRIKING RESULTS"

Referred to by Dr. Burnam were obtained by the administration of *Corpus Luteum* of the SOW, as presented in

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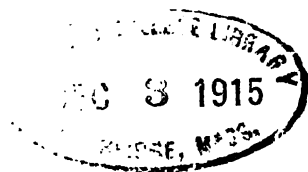
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THE EFFECT OF CHLOROFORM ON THE FACTORS OF COAGULATION

GEORGE R. MINOT, M.D.

From the medical service of the Massachusetts General Hospital

Received for publication September 27, 1915

That chloroform might accelerate the coagulation of blood was suggested to me by Dr. W. W. Palmer. He noticed that blood very rapidly became thickened and lumpy when he attempted to extract substances from it with chloroform.

Howell (1) attempted to use chloroform to isolate prothrombin. In so doing he noticed that a clear oxalated dialyzed plasma of a fasting cat when shaken with chloroform for one to two hours and filtered, clotted. He concludes from this that "prothrombin can be converted to thrombin in a calcium free solution," though in a less effective manner than by calcium salts. In order to investigate further what effect chloroform had on the factors of coagulation, the following observations were made:

To nine tubes each containing five drops¹ of clear oxalated guinea pig plasma, three, five and seven drops² of chloroform were each added to three tubes. The tubes were gently shaken to mix the chloroform with the plasma. A firm, very slightly cloudy, non-retractile jelly clot was formed in each tube. The time in which the clot formed varied from 30 to 60 minutes, even in the tubes containing the same amount of chloroform. If fresh blood platelets were added to the plasma chloroform mixture the clot became somewhat retracted.

If from 3 to 7 drops of chloroform were added to the plasma and then an optimum amount of calcium chloride solution 0.5 per cent

¹ 1 drop of plasma measured approximately 0.05 cc.

² 1 drop of chloroform measured approximately 0.01 cc.

added immediately or after an interval of 15 to 30 minutes a clot appeared in 6 or 7 minutes. However, when the same amount of calcium chloride but no chloroform was added to the same plasma a clot did not appear until 10 minutes.

This experiment was repeated many times using human, rabbit, dog, cat and guinea pig oxalated plasmas with widely varying results, which did not depend upon the animal from which the plasma was obtained. The oxalated plasmas used had not clotted of their own accord at the end of 48 hours. Sometimes a plasma would clot when chloroform was added to it in as short a time as 20 minutes, again not for 6 to 24 hours and quite as often not at all. A plasma that had been standing over 12 hours seemed to clot upon the addition of chloroform more often than a fresh plasma. Whether 3 or as many as 12 drops of chloroform were added to the 5 drops of plasma seemed to make little difference. Usually if less than 3 drops of chloroform were used there was either no clot or a weaker clot appeared in a longer time.

The clots were usually solid, allowing inversion of the tube, although they sometimes were but sliding jelly. They were, however, not as firm as normal blood clots or clots of oxalated plasma with calcium.

The addition of chloroform to the plasma caused a slightly cloudy opalescence. Sometimes there would also appear a few fine white flocculi, usually only in the tubes with the greater amounts of chloroform. These flocculi would settle with the chloroform to the bottom of the tube and their presence did not affect the clotting time or the nature of the clot.

If the chloroform plasma mixture was shaken very violently it became the consistency of wet sticky snow. This may have been due to a colloidal suspension, for it could be broken up by alcohol.

The effect of larger amounts of chloroform will be spoken of later.

In order that certain plasmas to which chloroform had been added might clot, it was found that the chloroform must be well mixed with the plasma; and also after mixing, the tube must be kept still, because a very little motion delayed markedly the time in which the clot formed as well as its firmness. Even with these precautions, however, many chloroform plasmas failed to clot.

Various oxalated plasmas were dialyzed in celloidin sacs against salt solution to remove the excess of oxalate. Those plasmas which clotted after adding chloroform before dialysis clotted afterwards and those not clotting before did not clot afterwards. Thus variations in the amount of oxalate present was not the cause for the varying action of chloroform.

When an optimum amount of calcium and a few drops of chloroform were added to those plasmas which clotted in the presence of chloroform alone, a clot usually formed in a shorter time than when only calcium was added. Plasmas not clotting upon the addition of chloroform sometimes clotted upon the addition of chloroform and calcium in the same amount of time as which they did with calcium alone. At times a slight acceleration occurred; while occasionally there was a slight delay, usually when the larger amounts of chloroform were added.

In order to decide if prothrombin could be converted to thrombin by chloroform, the following experiment was done and repeated:

FIBRINOGEN ¹ DROPS	CaCl ₂ (0.5 PER CENT) DROPS	CHLOROFORM DROPS	PROTHROMBIN ² DROPS	REMARKS
10	3			No clot in 24 hours.
10	3		5	Clot in 8 minutes.
10			5	No clot in 24 hours.
10		Amts. varying from 1 to 15 drops.	5	No clot in 24 hours.
10	3	Amts. varying from 1 to 15 drops.	5	Clot in 7-10 minutes.

¹ Pure solution made by modification of Hammarsten's method.

² Several solutions made according to Howell's method. *American Journal of Physiology*, xxxv., 474, 1914.

This experiment shows that chloroform was unable to convert prothrombin to thrombin while calcium did so readily.

Various attempts were made to determine the presence of thrombin and fibrinogen in the serum from the clots formed by adding chloroform to plasma, but neither was found.

The effect of chloroform on thrombin, fibrinogen and antithrombin was next studied.

Chloroform was thoroughly mixed with an equal and with half the amount of a pure solution of thrombin.³ This caused a slightly opalescent solution but no precipitate. This thrombin acted fully as well as a control specimen at once or after several hours, showing that chloroform did not affect thrombin.

³ From crystals of thrombin made according to Howell's method. *Am. Jour. of Physiol.*, xxvi, 453, 1910, and xxxii, 284, 1913.

Three to ten drops of chloroform mixed with eight drops of a solution of fibrinogen plasma,⁴ or with a solution of pure fibrinogen, and allowed to stand 1 minute to 60 minutes did not weaken their ability to be clotted by thrombin; provided the chloroform did not permit the fibrinogen plasma used to clot within 60 minutes. The pure fibrinogen solution never clotted upon the addition of chloroform.

In fact the chloroform fibrinogen plasma solution clotted with thrombin slightly faster than the control fibrinogen plasma. This may be explained by the effect of the chloroform on the antithrombin (discussed below) contained in the fibrinogen plasma.

That chloroform destroys or renders inactive antithrombin is shown by the following data in Table I obtained from repeated experiments.

TABLE I

THROMBIN DROPS	ANTI-THROMBIN ¹ DROPS	CHLOROFORM DROPS	INTERVAL OF TIME (MINUTES) AFTER THROMBIN ADDED BEFORE FIBRINOGEN SOLUTION ADDED	FIBRINOGEN SOLUTION DROPS	CLOT IN MINUTES
2	0	0	0 or 15	8	3
2	0	3 or 5	0 or 15	8	2½
2	1	0	15	8	28
2	1	2, 3 or 6 drops well mixed with antithrombin for 1 min. to 1 hr. before thrombin added.	2, 15 or 30	8	3-5
2	1	Thrombin and antithrombin mixed for 15 min., then 2, 3 or 6 drops added and well mixed.	2 or 15	8	4-7
2	1	3 drops without mixing.	15	8	18
2	1	¼ drop well mixed.	15	8	15
2	1	10 drops not mixed.	15	8	5

¹ For discussion of antithrombin test see paper by Minot and Denny in print for Archives of Int. Med.

It was found that in order to have the chloroform render the antithrombin ineffective the two must be well mixed.

Ether was found to act similarly to chloroform. Like chloroform its presence sometimes allowed an oxalated plasma to clot and was

⁴ From dried specimens of plasma made according to Howell's method. Archives of Int. Med., xiii, 76, 1914.

able to render ineffective antithrombin. In contrast to chloroform, however, the few observations made showed that ether always delayed the clotting of an oxalated plasma with calcium, causing often only a weak clot to form, and unlike chloroform it would slightly weaken the power of a solution of thrombin.

Carbon bi-sulphide, petroleum ether, benzol, xylol, potassium chloride, magnesium sulphate, calcium chloride and weak alkalies were found to have no inhibiting power on the action of antithrombin. Dilute weak acid, however, did inhibit the action of antithrombin, as has also been pointed out by Collingwood and MacMahon (2).

Doyon (3) and also Billard (4) have been able to isolate from the liver and other organs by a chloroform extraction process an antithrombin thought to be of a nucleo-protein nature. It is probable that this substance is not the same as Howell's antithrombin of the circulating blood, for Doyon is able to carry it through certain procedures such as heating to high temperatures that would destroy Howell's antithrombin.

Jobling and Petersen (5) have shown that chloroform and ether may remove the antitryptic power of serum, which is probably due to compounds of unsaturated fatty acids. They were able to recover the antitrypsin from the extracts by saponification. It takes several days' extraction at room temperature to remove completely the antitryptic power of serum while antithrombic power is removed at once.

Attempts were made to see if Howell's antithrombin could be recovered from chloroform extracts of the blood, for it was thought that it might be similar to Doyon's antithrombin or to antitrypsin. To a solution of antithrombin (oxalated plasma heated to 60°C. and filtered) varying amounts up to twice as much chloroform were added and the mixture centrifuged. The opalescent solution free from chloroform in the upper half of the tube exhibited very feeble antithrombic power as compared to the control. The material at the bottom of the tube with the chloroform, after removal of the chloroform exhibited no antithrombic power, while Doyon's extracts of antithrombin from organs did. Other attempts with serum and with plasma unheated and heated to 60°C. have been unsuccessful so far in recovering Howell's antithrombin from chloroform or ether extracts. However, a further study especially on the effect of complete saponification of such extracts is necessary before we can say whether Howell's antithrombin like antitrypsin is absorbed by chloroform and ether or whether it is destroyed. Serum antitrypsin can be absorbed by starch.

From my observations various experiments have shown that starch has no effect on antithrombin.

Zak (6) has shown that petroleum ether extracts of the red blood corpuscles contain a substance which retards coagulation. I have been unable to obtain with chloroform extracts of the red cells any substance with antithrombic power.

Large amounts of chloroform precipitated fibrinogen and prothrombin from oxalated plasma, but no material was obtained with antithrombic power. An equal amount or more of chloroform added to oxalated plasma, well shaken and centrifuged, caused a thick creamy white precipitate to form as a band in the middle of the tube. This precipitate was soluble in normal salt solution. It clotted with calcium and also thrombin, showing that it contained prothrombin and fibrinogen. No antithrombin could be demonstrated in a solution of this precipitate. In one instance a solution of this precipitate clotted by itself after 18 hours as a weak, water-clear clot, showing that some thrombin was present. In three other instances no clot occurred in 24 hours. In the centrifuge tube above the precipitate there remained a clear solution (A) equal in volume to the faintly cloudy solution (B) below the precipitate. Neither A nor B had any antithrombic power.

Small amounts of prothrombin and fibrinogen were usually found in A, rarely in B.

Equal amounts of ether and plasma acted similarly to chloroform and plasma, although ether caused a smaller precipitate.

Whether a trace of free thrombin exists in the circulating blood is not known, but we do know that thrombin begins to be formed from prothrombin as soon as the blood is shed. Hence the speed and method with which blood is collected may be factors in determining how much free thrombin exists in an oxalated plasma.

The blood from which the clear plasma for the above experiments was obtained was drawn from the heart or big veins of animals and veins of humans with an all glass syringe previously sterilized and rinsed in salt solution. The blood was at once mixed with an oxalate solution (0.1 per cent sodium oxalate in 0.9 per cent salt solution). In some instances the oxalate was placed in the barrel of the syringe so that the blood became mixed with the oxalate as it was drawn. With such procedures, more especially the former, it is probable that a trace of free thrombin will occur in the plasma.

Finding that antithrombin was rendered inactive by chloroform it seemed that perhaps the reason why some oxalated plasmas clotted

upon the addition of chloroform was that some contained more free thrombin than others; so that after the chloroform had rendered inactive the antithrombin, the free thrombin could clot the fibrinogen. This would explain the results obtained in the above experiments.

In order to test this point, the following procedures were undertaken: Blood was collected with a paraffined cannula from the carotid artery of 4 cats and 2 rabbits and run directly into oxalate solution. The fresh clear plasma from these animals did not clot upon the addition of chloroform. Of five plasmas similarly obtained from veins one clotted feebly upon adding chloroform. Clear plasma obtained from blood, which had been allowed to remain a few minutes in a glass syringe before being mixed with oxalate solution, did not clot by itself. Such plasma did, however, almost always clot when chloroform was added to it.

Therefore it seems that the reason that chloroform permitted some oxalated plasmas to clot, which did not clot themselves, is that it renders inactive the antithrombin and allows any free thrombin to clot the fibrinogen.

From this it would appear that the addition of thromboplastic substances which neutralize antithrombin ought to clot similar oxalated plasmas in a similar manner as chloroform. It was found that a solution of kephalin⁵ (the active thromboplastic material) in sufficient amount did.

A rabbit with typical acute chloroform poisoning from 1 cc. of chloroform in oil given subcutaneously 48 hours before showed a diminished amount of antithrombin. As the total amount of chloroform injected was small and as chloroform is broken up in the body, it does not seem likely that the low antithrombin content was due to the action of the chloroform on the circulating antithrombin. The liver is important in the formation of antithrombin and Denny and Minot (7) have shown that in dogs with liver destruction from phosphorus poisoning the antithrombin in the blood may be markedly diminished. Though chloroform produces destruction of a different part of the liver than phosphorus perhaps the decreased antithrombin in the rabbit was due or partially due to destruction of the liver tissue. Acidosis may perhaps be an added cause. Acidosis⁶ occurred in the chloroform poisoned rabbit and it not infrequently occurs in phosphorus

⁵ Prepared by Howell's method. *Am. Jour. of Physiol.*, xxxi, 1, 1912.

⁶ Tested by method described by Rowntree, Marriott and Levy. *Transactions of 13th Annual Meeting of the Association of American Physicians*, 1915.

poisoning⁷ and has been found to occur in other experimental and some clinical conditions where the antithrombin was low. The effect of acidosis on antithrombin will be discussed in another paper.

SUMMARY

1. Antithrombin is rendered inactive by chloroform and ether, thus allowing free thrombin if present in an oxalated plasma to clot fibrinogen.
2. Prothrombin is not converted to thrombin by chloroform.
3. Chloroform can precipitate both fibrinogen and prothrombin from an oxalated plasma.
4. Chloroform does not weaken the action of a solution of pure thrombin. Ether does slightly.
5. Antithrombin could not be recovered from chloroform or ether extracts of serum or plasma, unheated or heated to 60°C., and is not exactly identical to antitrypsin or to Doyon's antithrombin.
6. In one chloroform poisoned rabbit the antithrombin of the blood was decreased below normal.

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⁷ L. G. Rowntree. Personal communication.

PERISTALSIS AND COORDINATION IN THE EARTHWORM

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It is our purpose to analyze the normal progressive movements of the earthworm (*Lumbricus terrestris*) with a view to determining the physiological factors involved.

According to the descriptions of Friedländer (1) and of Biedermann (2) the progressive movements of this animal are brought about by a passive elongation proceeding from the anterior end. This is succeeded by a forcible extension which travels posteriorly. The third and last phase is an active shortening due to contraction of the longitudinal muscles. The peristaltic wave travels backward and as a result the animal moves forward since the setae are directed backward and, in the contracted portion, act as hold-fasts.

The origin of the peristaltic waves which cause the earthworm to move forward, has been ascribed by Friedländer to traction; Biedermann agrees with Friedländer and maintains that the initiation of peristalsis is due to a stretching of the myodermal sheath, and not to contact with a rough surface, for the same regular peristalsis may occur in a worm suspended in air. That it was the tension on the myodermal sheath with which these authors were concerned, and that the pull on the nerve cord was not responsible for the initiation of peristalsis, we have determined by dissecting the nerve cord free from surrounding tissues and applying varying degrees of traction to it alone; no peristalsis resulted; but immediately the animal was pulled by the body wall sufficiently to move it over the surface on which it rested, peristalsis began.

While it is clear that tension on the myodermal sheath plays an important rôle in the initiation of peristalsis, the underlying mechanism

¹ We wish to acknowledge our indebtedness to the management of the Marine Biological Laboratory at Woods Hole for providing facilities for carrying on this work.

by which tension produces such a result, is not clear, and it is certain that former experimenters have overstepped the mark in excluding all effects of surface stimulation. It therefore has seemed to us that even tension effects might be further analyzed on the basis of sensory impulses.

To test the reasonableness of this suggestion, we obtained graphic records of the effects of mechanical stimulation in the following way. A worm preparation was clamped near the posterior end and the anterior upper end was attached to a recording lever.² In order to exclude the possibility of any tension effects, the stimuli were applied below the clamp. If such a preparation be stimulated, when quiescent, by touching with a moist camel's hair brush, peristaltic waves as a rule start.

In different tests it was found that groups of peristaltic waves could be started by tapping. This result frequently succeeded only when several such stimuli were applied at regular intervals (fig. 1, 12, 10, 8). If instead of being quiescent, the preparation showed regular peristalsis, repeated tapping causes a marked acceleration, as noted in figure 1-x, in which case the rate of rhythm increased from six to twelve per minute. These results show that our assumption was correct and that even a regular peristaltic rhythm may be initiated by sensory stimulation. This led to an attempt further to analyze the effects of sensory stimulation.

THE EFFECTS OF SENSORY STIMULATION

If a worm preparation or an intact worm be sharply touched at any point the result is an immediate shortening throughout the entire length of the worm; a reaction which in no way simulates any phase of peristalsis. A light touch or a gentle pinch elicits a response of an entirely different nature; applied to the anterior end, in addition to the localized shortening, an active lengthening takes place and the animal moves away from the point of stimulation. This extension may involve the entire animal. If, however, the stimulus is applied to the posterior end there is a shortening throughout the entire extent of the preparation, somewhat more marked at the point of stimulation.

² In this work, unless otherwise specified, a 'worm preparation' was made by cutting off both the anterior and posterior ends of an individual so as to provide a middle portion of suitable length. In clamping, a modified Gaskell clamp was used and the preparation sufficiently compressed to hold the part firmly without impairing the conductivity of the nerve cord.

The animal is pulled forward, since the setae act as hold-fasts, being extruded coincidentally with the contraction of the longitudinal muscles.

It is evident that the direction of propagation of the impulse determines the nature of the response, whether it be a lengthening or a shortening, and that stimulation somewhere in the mid-course of the preparation should determine different events anterior and posterior to the point of stimulation.

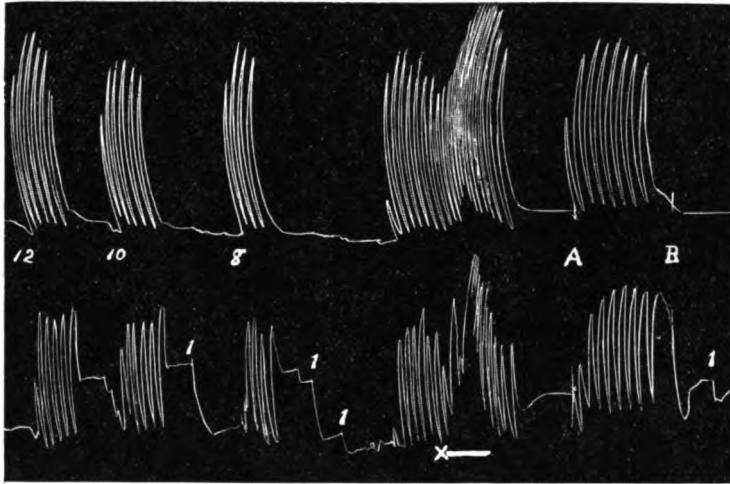


Fig. 1. Upper tracing of anterior half and lower tracing of posterior half of a worm preparation lightly clamped near the mid point. At each of the points marked 1 a single touch stimulus was applied just above the clamp and caused elongation of the posterior half. The figures 12, 10 and 8 represent number of contact stimuli applied to the posterior half near the clamp at the rate of about one per second. The group of contractions above x was spontaneous and the rhythm was accelerated by repeatedly touching the posterior piece for one minute, as indicated by the horizontal line. At A the anterior half was stroked beginning at its anterior end. At B same half was stroked from the clamp back to the anterior end.

It was a simple matter to devise a method of obtaining a graphic record and analyzing in detail these phenomena of shortening and lengthening. The worm preparation was firmly clamped in the middle, as described above, without impairing the conductivity of the ventral nerve cord. The free ends were attached to levers recording simultaneously on a smoked drum. When a light mechanical stimulus was applied immediately above the clamp a marked shortening of the whole

anterior portion took place and was attended by a simultaneous lengthening of the whole posterior portion below the clamp (fig. 1.) This usually started a peristaltic wave which began at the anterior end of the preparation and which traveled through and involved the posterior part. It would seem then that a peristaltic wave may begin with a shortening and does not necessarily involve a preliminary extension of the initiating part (Biedermann, loc. cit.) Weak faradic stimulation applied to a point on the surface of the animal gave the same result as mechanical stimulation. In other words, local stimulation gives a picture which is identical with peristalsis as seen in the intestine of higher animals, viz., contraction above and relaxation below the point of stimulation.

These results explain an observation which we have made on the effects of stroking the surface of a suspended preparation with a moist brush. If the strokes be from the anterior to the posterior end, peristaltic waves are set up due to the fact that there is a shortening anterior to the point stimulated (fig. 1, A). If the direction of the stroke is reversed, and is made from the posterior towards the anterior end of the preparation, peristalsis is inhibited and the animal remains extended (fig. 1, B). Sometimes by repeated upward stroking complete quiescence in the extended state could be obtained, at other times the duration of quiescence corresponded to that of several peristaltic waves. We also found that the normal rate of peristalsis is always accelerated by downward stroking with a camel's hair brush; for example, in one preparation with the lower end weighted, giving 10 to 12 peristaltic waves per minute, the rate was increased to 16 by stroking downward. In the same preparation peristalsis was completely inhibited for 30 seconds by upward stroking, and when the weight was removed upward stroking caused extension with complete quiescence.

The results of this experiment may be applied to the explanation of the coordinated creeping movements of a worm preparation when drawn over moist filter paper, anterior end foremost (Friedländer) (3). In terms of sensory stimulation of the worm surface, the forward pulling is equivalent to backward stroking, and therefore sets up a series of peristaltic waves. If now, the worm preparation be drawn backward posterior end foremost over damp filter paper, shortening and peristalsis is inhibited, the piece simply extends and if care be exercised to avoid acute stimulation or overextension the piece may remain quietly extended for a long time. This effect is readily understood, for backward pulling over a rough surface produces a sequence of sensory effects

similar to those due to upward stroking with a brush. From these results it follows that when a worm creeps forward each peristaltic wave will tend to involve the whole worm and will be followed by another; thus progression continues until some other opposing factor, such for example as central fatigue, is interjected. If on the other hand a backward movement should take place, the sequence of the stimuli tends to bring the animal into a state of rest which will persist until the animal is again definitely excited to peristaltic activity.

Moreover, the fact that peristaltic waves may be set going by causing shortening of several segments, as is the case in a downward stroke in which more and more of the worm is anterior to the point of stimulation, would again indicate that peristalsis can be initiated by a contraction of the longitudinal muscles. Such a conclusion is further sustained by the fact that upward stroking, in which more and more of the worm lies posterior to the point of stimulation and therefore extends, inhibits peristalsis and may leave the worm extended and quiescent. Clearly extension does not necessarily initiate peristalsis.

In view of these facts it seems reasonable to suppose that the effects of traction in bringing about peristalsis in a preparation hanging free in the air are to be accounted for on the assumption that the stretching of the skin causes sensory impulses to be set up. These impulses cause reflex shortening anteriorly and lengthening posteriorly. This would also account for the fact that when a worm preparation is hung up by either the anterior or posterior end, the peristaltic wave with rare exceptions begins at the anterior end.

Since sensory stimuli (contact, or traction), acting upon the earthworm tend to set up peristaltic movements, we may, with right, raise the question whether all normal peristaltic movements may not be the result of sensory stimuli.

THE RÔLE OF THE VENTRAL NERVE CORD

The differences in the effects produced anterior and posterior to the point of stimulation, still further resolves itself into a question whether they be due to differences in the character of the stimuli or to the intermediation of the central nervous system. In order to put this question to the test we have studied the effects of stimulation of the nerve cord itself. After anaesthetizing a worm preparation in a solution of ether in water, portions of the nerve cord at anterior and posterior ends was dissected free from surrounding tissue. The responses of these portions of nerve cord to selected faradic stimulation are identical with those

elicited by mechanical cutaneous stimulation described above, but the responses may vary with the strength and duration of the stimulation as noted in the following experiments.

Two centimeters of the dorsal myodermal sheath and intestine were removed near the middle of an anaesthetized worm preparation. The nerve cord was exposed and carefully separated from the ventral portion of the body wall. The latter strip was left connecting anterior and posterior sections of the worm, and was made fast to a cork plate by four pins. The nerve cord was kept moist with Ringer's solution, and was known to be uninjured by the fact that, after recovery from the anaesthetic, peristaltic movements in the posterior piece of the worm

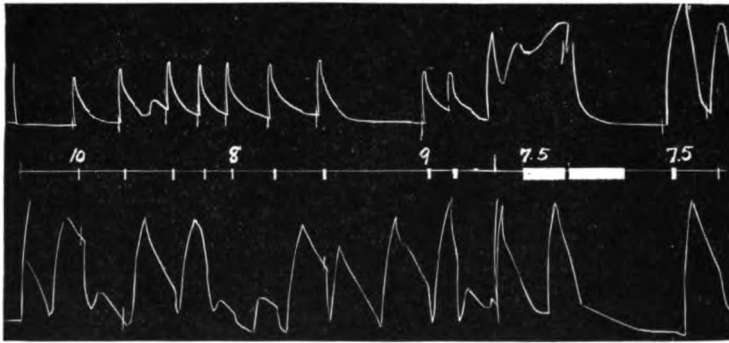


Fig. 2. Effects of faradic stimulation of the nerve cord in the middle of the worm preparation. The upper tracing records events of the anterior half and lower tracing of the posterior half of the preparation. The stimulation periods are recorded on the middle line. The figures represent the strength of the stimuli in terms of centimeters to which the secondary coil has been withdrawn from the primary.

followed in regular sequence those which began in the anterior segments. In this case the portion of the ventral musculature did not conduct the impulses for the contraction wave did not extend through it and the impulses were still conducted when the cut extended through this connection but left the nerve cord intact. On other preparations the ventral bridge of muscle and skin was left intact but the coordination of the posterior with the anterior portions of the preparation ceased immediately upon cutting the ventral nerve cord. We thus effectually and conclusively proved the correctness of Biedermann's contention that the peristaltic impulses are conducted by the nerve cord.

If a small cork plate were used in pinning the above preparation it could be clamped to a stand, and graphic records of the contractions of the anterior and posterior ends of the worm could be easily obtained by attaching them to separate levers. By means of a thread loop the isolated section of the nerve cord was slightly raised and brought to rest upon a pair of platinum electrodes which were carefully kept from contact with any other tissue. Faradic shocks were obtained by the use of a Porter inductorium. If the nerve cord was stimulated with appropriate shocks (with the coil at 10 to 8 cm., fig. 2) the anterior section shortened,³ whereas, the posterior lengthened at first and subsequently shortened in response to the impulse propagated back from the anterior half. Shocks continued for two minutes with the coil at 7.5 cm. produced a tonic contraction of the anterior part and a prolonged lengthening of the posterior part (fig. 2). Stronger but shorter stimulation resulted in some cases in rapid rhythmical peristalsis of the posterior part and persistent contraction of the anterior part of the preparation. The experiment proves that sensory stimuli, do not determine the character of the muscular response by virtue of their nature, but that the responses are determined by the direction the impulses travel in the nerve cord, and ultimately by the connection and the orientation of the elements in the central nervous system. These findings harmonize the coordination of the movements of the earthworm with the analyses made for crustacea by Loeb and Maxwell (5) and for the vertebrates by Loeb and Garrey (6).

While the responses described above harmonize perfectly with those obtained from mechanical cutaneous stimulation, and can be elicited at will by selecting the proper strength of faradic stimulation of the nerve cord, they are not by any means invariable, for changing the strength of the stimuli may change the reaction; nevertheless, one can always predict the character of the response. In a quiescent aboral preparation the nerve cord at its anterior end was stimulated. With the secondary coil at its maximum distance from the primary and inclined at an angle of 45°, stimulation for one second caused only a slight localized twitch. At the angle 30°, half of the preparation nearest the electrodes twitched, while at 15° the whole preparation gave a twitch followed by a peristaltic wave. Increasing the duration of the weaker stimulation caused the initial twitch, persistent shortening of the more anterior segments, and a peristaltic rhythm. With the

³ A. J. Carlson (4) observed shortening anterior to point of stimulation in *Bispira*.

secondary coil parallel to and 12.5 cm. from the primary, the whole preparation remained shortened during the period of stimulation—a result which can be due only to a preponderant contraction of the longitudinal musculature. With still stronger stimuli (10 cm.) there was invariably a writhing and squirming of the preparation, sometimes followed by peristalsis. Faradization stronger than 7.5 cm. caused a striking reversal in the nature of the reaction: all writhing and peristalsis ceased and the preparation became quiet in extreme extensions.⁴

The squirming was due to combined irregular contraction of both circular and longitudinal muscular coats; while the extension was due to relaxation of the longitudinal and contraction of the circular coat. Although the absolute strength at which these different results were obtained varied with individual preparations with recovery from anaesthesia and with fatigue due to stimulation of the nerve cord, the relative effects of weak, moderate and strong stimulation could always be elicited.

MUSCULAR PHENOMENA

So far we have referred to the phases of peristalsis only as a lengthening and shortening. Obviously the shortening may be due to a contraction of the longitudinal muscles, and the lengthening either to a relaxation of all of the musculature or to a contraction of the ring muscles accompanied by a relaxation of the longitudinal ones.

The action of the circular musculature can easily and clearly be demonstrated if the myodermal sheath be slit along the median dorsal line. In an otherwise normal worm in which a 3 or 4 cm. slit of this sort had been made, an observation of the progressive movements showed the elongation phase of peristalsis to be due to a double process, viz. the relaxation of the powerful longitudinal musculature and the contraction of the weaker ring muscles of the corresponding segments. In consequence the dorsal slit widened in spite of the decreased diameter of the animal in this region. The relaxation of the longitudinal muscles accompanying the contraction of the circular set is a beautiful and striking example of 'contrary' or 'reciprocal' innervation. The succeeding shortening of the segments is due to a contraction of

⁴ Stimulation of the nerve cord was never seen to produce squirming of the portion anterior to the point of stimulation. This, with the added fact that strong shocks produce quiescence and extension while more moderate stimulation produces violent squirming excludes these movements from the criteria of pain and confirms the analysis of pain sensations made by Norman (7).

the longitudinal musculature accompanied by a relaxation of the ring muscles of the segments involved. The relaxation phenomenon is clearly shown by the closure of the slit in spite of the increased diameter of the segments in question. Here again we have 'contrary' or 'reciprocal' innervation in the opposite sense to that considered above.

These phenomena have been duplicated for the greater part by electrical stimulation of the nerve cord exposed in the fashion already described. Stimulation (coil at 9 cm.) gave a slow simultaneous even widening of the slit for a considerable distance posterior to the point of stimulation, with no contraction of the longitudinal muscles. The result was therefore a lengthening of the posterior part. At the same time the portion anterior to the exposed nerve shortened because of contraction of the longitudinal muscles but at the same time showed contraction of the circular ones, a difference from the normal which is probably due to the strength of stimuli used.

PROPAGATION OF STIMULI AND FATIGUE

No account of the normal reactions of the worm would be complete without an added reference to the propagation of stimuli of different strengths and to the fatigue phenomena which develop with repeated reaction to stimuli.

Some effects of varying the strength of stimulation of the nerve cord have been noted in a previous section; to these may be added those which are constantly obtained in experiments of the following type. In one preparation, faradic stimulation for one second with the coil at 7.5 cm. caused only 25 segments to respond; at 4 cm. a response was elicited from 50 segments while at 0, 90 segments, i.e., the whole worm preparation, contracted. It is clear from results such as these that the intensity of the response decreases with distance from the point of stimulation, and the strongest response occurs at that point. The relation of this fact to the local contraction which always occurs as the result of mechanical or electrical stimulation is obvious. Stimulation, either mechanical or faradic, of the cutaneous surface gave similar results, the proximate segments being more intensely affected. No attempt was made to eliminate direct stimulation of the musculature.

After the nerve cord had been stimulated repeatedly with faradic shocks of a given strength, the muscles ceased to respond. With an increase in strength of the stimulus there was a renewal of the muscular response. Since cutaneous stimulation gave a local response, the rise

of threshold in this experiment indicated central nerve fatigue. Furthermore, a stimulus of moderate intensity causes a muscular response of a certain number of segments, which, after continued stimulation, no longer responded to this stimulus. When the intensity of the stimulus was slightly increased only a limited number of proximate segments showed contraction of the musculature. The failure of the stronger stimuli to involve the musculature of the more distant segments, which had at no time shown contraction, again proves that the ventral cord had been fatigued. Reference has already been made to the possible relation of this fatigue to the suppression of progressive movements of the animal.

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THE CARDIO-INHIBITORY CENTER

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The cardio-inhibitory center in the medulla oblongata has not yet been definitely localised. Laborde (1), who attempted to do so, employed the method of puncture (*piqûre*) and obtained inhibition from a point situated, according to his drawing, in the lateral part of the fourth ventricle some distance cephalad to the dorsal vagus nucleus. He appears to have believed that the cardio-inhibitory fibers originate in the nucleus ambiguus, which he refers to by the designation "noyaux accessoires de l'hypoglosse et des nerfs mixtes (pneumogastrique, spinal, glossopharyngien)." His observations were made on cats and dogs.

Schaternikoff and Friedenthal (2), in a study directed to ascertain in which medullary rootlets the cardio-inhibitory fibers emerge, applied unipolar faradisation with a needle electrode to the medulla. They made use of curarised rabbits. They did not determine the location of the inhibitory center and indeed obtained no cardiac slowing by stimulation of the surface of the fourth ventricle but only after plunging the electrode into the deeper layers of the medulla.

The histological researches of Kohnstamm (3) and the more extensive researches of van Gehuchten and Molhant (4) led these writers to infer that the cardio-inhibitory fibers arise in the dorsal vagus nucleus. Since this conclusion is at variance with the results of excitation referred to above we undertook the following experiments in the hope of deciding the question.

METHODS

In our experiments, which were performed on the dog, the animal was anaesthetised first with chloroform and ether and the narcosis was maintained by an intravenous injection of chloralose (0.2 per cent solution).

A median incision was made at the back of the neck and the muscles were detached from the skull and reflected so as to expose the occipital bone and the occipito-atloid ligament. The occipital bone was cut away with bone forceps almost, but not quite, as far forward as the transverse sinus, bleeding being checked by the use of wax. The caudal portion of the cerebellar vermis was then carefully removed so as to bring to view the floor of the fourth ventricle in the vicinity of the dorsal vagus nucleus. The ventricular floor thus revealed lies at the bottom of a deep well of structures and in order to obtain a satisfactory illumination of it an electric head-light was found necessary.

The spinal cord was divided at the level of the atlas vertebra, the lamina of this being first cut away. The object of the procedure was

to avoid the rise in blood-pressure which otherwise takes place on faradisation of the medulla. Following the section artificial respiration was at once instituted.

Unlike Schaternikoff and Friedenthal (2) we did not employ curare, owing to the fact that it raises the threshold of the cardio-inhibitory mechanism, a circumstance which is disadvantageous when accurate localisation is to be made. The drug, indeed, was not necessary because, with

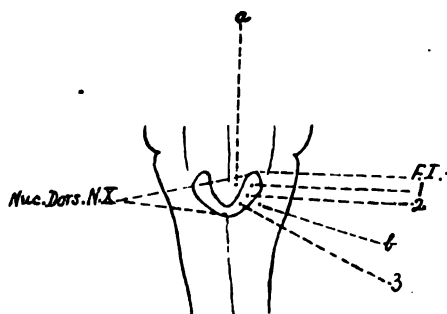


Fig. 1. Diagram of medulla oblongata of dog. Natural size. *F.I.*, inferior fovea; *Nuc. Dors. N.X.*, dorsal vagus nucleus; *a*, *b*, *1*, *2*, *3*, points at which faradisation was applied.

the weak currents used, no muscular movements were detectable beyond a very slight trembling of the head; movements of the trunk and limbs were, of course, precluded by the division of the cord. The currents used by Schaternikoff and Friedenthal must have been very strong and, as the cord was not cut, curare was necessary.

Faradisation was applied by the unipolar method, the stimulating electrode being of the Sherrington type for cortical localisation; the indifferent electrode was secured to a hind-limb. In order to obtain satisfactory responses we found it essential to keep the medulla warm by the application of hot, moist pads.

The blood-pressure in the carotid artery was recorded by a Hürthle manometer. The animal was secured with the abdomen resting on the table, the head being firmly clamped.

EXPERIMENTAL RESULTS

On examining the floor of the fourth ventricle the dorsal vagus nucleus (ala cinerea, trigonum vagi) is easily discernible by its grey, translucent appearance (fig. 1).

It is an elongated structure about 6 mm. in length, wider in front and gradually tapering backwards to meet its fellow of the opposite side. At its cephalic extremity there is a small depression, the inferior fovea.

The procedure in localising the effects in the nucleus was as follows: While the blood-pressure was being recorded stimulation was applied

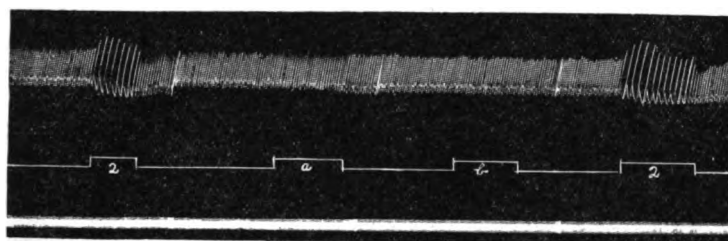


Fig. 2. Record of cardiac effects on stimulating points on medulla oblongata shown in figure 1. Sec. dist. 10 cm. Time in $1/5$ secs.

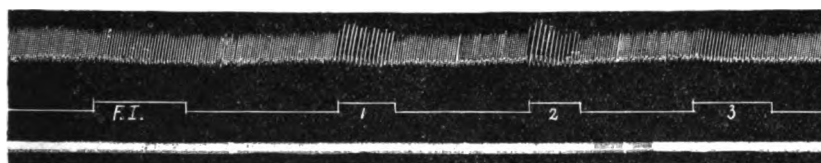


Fig. 3. Record of cardiac effects on stimulating points on medulla oblongata shown in figure 1. Sec. dist. 10 cm. Time in $1/5$ secs.

to the nucleus with a current intensity sufficient to evoke a definite cardio-inhibitory effect. The current was then reduced as much as it was possible to do and still obtain slight though definite inhibition from the nucleus. With this minimal current the surface of the ventricle in the neighborhood of the nucleus was explored. No inhibition was evoked mesially or laterally to the nucleus, even at points a millimetre or less from that; but upon returning to the nucleus inhibition was at once elicited. These facts are illustrated in figure 2, in which it may be seen that no effects were obtained from stimulating points *a* and *b* beside the nucleus but a distinct one from point *2* on the nucleus itself. These points are shown on the diagram of the medulla.

Having localised cardiac inhibition in the nucleus this was next interrogated from end to end with the electrode. The entire nucleus was observed to yield inhibition. Inhibition could also be evoked from the inferior fovea, although the response was invariably less pronounced than that from the nucleus itself. It is probable that the inhibition obtained from the inferior fovea is produced in part reflexly by stimulation of the sensory fibers of the tractus solitarius accessible at this point to the current. Stimulation of the inferior fovea was always accompanied by swallowing movements (5). The caudal part of the nucleus sometimes gave a weaker inhibitory effect than the cephalic part but, as this difference was not invariable, we do not attach much significance thereto. The effects of stimulation of the inferior

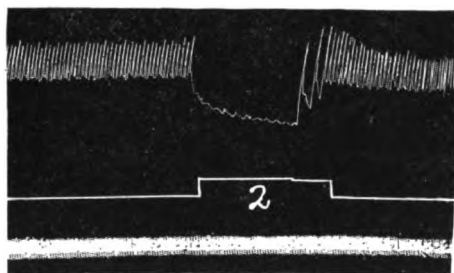


Fig. 4. Record of cardiac effect on stimulating point on medulla oblongata shown in figure 1. Sec. dist. 9 cm. Time in 1/5 secs.

fovea and various points on the nucleus are shown in figure 3. The points referred to in the record are indicated on the drawing of the medulla; they were selected at random and others in their neighborhood yielded similar results.

With currents slightly stronger than those used for localisation complete inhibition can be elicited from the dorsal vagus nucleus.

This is shown in figure 4 in which stoppage of the heart occurred on stimulation of the middle point of the nucleus.

Laborde (1) claimed to have excited inhibition by puncture of the medulla at a point considerably in front of the dorsal vagus nucleus; but he does not appear to have attempted any very definite localisation and, in fact, such would be impossible with his procedure. His impression that the cardio-inhibitory center is in the nucleus ambiguus is contrary to the results obtained by histologists and by us.

Schaternikoff and Friedenthal (2) asserted that they were unable to excite inhibition when the electrode was applied to the surface of the fourth ventricle and only succeeded in doing so after thrusting it into the substance of the medulla. They also stated that inhibition was readily evoked from the terminations of the posterior columns.

We have found, on the contrary, that when the medulla is exposed carefully it is always possible to obtain inhibition from the surface of the dorsal vagus nucleus with weaker currents than from the posterior columns. Schaternikoff and Friedenthal make no mention of the currents they employed but presumably they were strong since with weak ones they could scarcely have got inhibition from the interior of the medulla. Their failure to evoke inhibition from the floor of the fourth ventricle was probably due to injury in the preparation or to their not having kept the medulla warm. The latter point we found to be most important since, if the medulla becomes cooled, the response is lost.

SUMMARY

The results of our experiments show that the cardio-inhibitory center is situated in the dorsal vagus nucleus and are thus in harmony with the histological investigations of Kohnstamm and of van Gehuchten and Molhant.

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THE EFFECTS OF AQUEOUS EXTRACTS OF ORGANS UPON THE CONTRACTIONS OF UNSTRIATED MUSCLE FIBERS

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Our first communication dealt with an aqueous extract of the thyroid gland. We tested the effects of the different substances which can be separated from it upon the heart action, respiration and blood pressure in dogs, and recorded the results in the usual kymograph tracings. We found that the nucleo-proteins, globulins and coagulable proteins, when administered intravenously in dogs, showed no demonstrable activity. But the filtrate which remains after the removal of these substances exhibited great activity. It produced a noticeable and immediate fall in blood pressure, together with a slight coincident deepening of respiration. But it did not produce any appreciable acceleration of the heart's action. This filtrate was divided into two portions by evaporating to dryness and taking up with 95 per cent alcohol. This gave "alcohol soluble" and "alcohol insoluble" portions. The former was found to manifest the same activity in approximately the same proportion as the original first filtrate which remains after the removal of the nucleo-proteins, globulins and coagulable proteins.

A further separation was made by adding basic lead acetate to the "alcohol soluble" portion until no further precipitation occurred. This yielded what we have termed our "lead precipitate" and "lead filtrate" portions. The "lead filtrate" proved to be the active portion and the richer in iodine. We have retained and used the first filtrate (which remains after the removal of the nucleo-proteins, globulins and coagulable proteins) under the designation of the "thyroid residue."

All of the parts of an aqueous extract of the thyroid which we have tested contain iodine in different amounts. An extract of the pig's thyroid is much richer in iodine than that of the sheep. When the ni-

trogen content of a dose of pig thyroid is made the same as that of a sheep residue, the sheep residue shows less activity in the kymograph tracings than the pig. But when the iodine content of the dose of each residue is made the same, the effect of each upon blood pressure are approximately the same. Hence, the vaso-dilating principle which is present only in the "residue" portion of an aqueous extract of the thyroid is not contained in an active form in all of its iodized proteins, but it exists in some particular, iodized molecule. This is to be sought in the "alcohol soluble" part and the "lead filtrate" from the original entire residue.

Our second communication dealt with the similar substances which can be isolated from aqueous extracts of the pituitary, parathyroid, thymus and adrenal glands and from the pancreas, liver and muscle. We found that the residue portion of these extracts, or the filtrate which remains after the removal of the nucleo-proteins, globulins and coagulable proteins, was the only portion which showed activity in the kymograph tracings. Each "residue," with the single exception of that of the adrenal gland, exhibited a peculiar and definite vaso-dilating power which, when the dose of residue was standardized by its nitrogen content, seemed to be characteristic of the organ from which the residue was derived. The residue from the whole adrenal gland showed a marked vaso-constrictor effect, but the kymograph tracing shows quite a different curve from that produced by the usual 1-1000 solution of commercial adrenalin. From these investigations it has seemed reasonable to conclude that every organ produces some active substance which can be found only in the residue of the organ from which it is derived, and is "active" in the sense that it produces through the circulation some demonstrable physiological change in one or more other organs.

The present communication is an attempt to confirm the location of the active principle of every organ in the residue part of its aqueous extract, and at the same time to ascertain something of its physiology. The simplest and most direct experiments are those with smooth muscle fiber.

A small strip of this tissue was removed from different portions of the intestine and from the uterus of a freshly killed cat, and suspended from the short arm of a writing lever, in a cylinder containing 275 cc. of Locke's solution. This preparation was maintained at 38°C., by immersion in a vessel of water to which a small flame could be constantly applied. A large flask containing the Locke's solution, which

was constantly kept at 38°C., was arranged to syphon into the cylinder holding the suspended muscle segment, in order that the muscle could be washed and the cylinder refilled with warm fresh solution as often as might be required. By this method the dilutions of the different substances whose effects were to be tested could be calculated in terms of nitrogen computed as protein.

The fractions of the aqueous extracts which we have tested consist of the nucleo-proteins, globulins, coagulable proteins and the filtrate or residue which remains after their removal. The extracts of the pituitary and adrenal glands, like those of the others, were each made from the entire organ. The "alcohol soluble" portion of the thyroid residue in the previous kymograph tracings seemed to contain *all* of the active material which exists in the original residue, but in these muscle tracings was not as active as the *whole* thyroid residue.

TEST SUBSTANCE	AMOUNT OF NITROGEN COMPUTED AS PROTEIN MGS. PER CC. SOL.	IODIN MGS. PER CC. SOL.
Thyroid residue.....	26.15	0.2
Alcohol soluble part.....	16.8	0.2
Alcohol insoluble part.....	27.	0.1
Thyroid globulin.....	24.	0.025
Thyroid coagulated protein.....	8.4	0.01
Thyroid nucleo-protein.....	6.1	0.0275
Thymus residue.....	41.3	0
Thymus globulin.....	1.1	0
Thymus nucleo-protein.....	12.7	0
Spleen residue.....	43.25	0
Spleen globulin.....	2.5	0
Pituitary residue.....	24.5	0
Pancreas residue.....	112.7	0
Pancreas nucleo-protein.....	2.5	0
Liver residue.....	45.	0
Alcohol soluble part.....	42.5	0
Liver nucleo-protein.....	7.2	0
Adrenal residue.....	22.6	0
Adrenal nucleo-protein.....	4.2	0
Adrenal globulin.....	3.5	0
Parathyroid residue.....	16	0

The amount of the substance which was tested was computed by the nitrogen content in terms of protein, and was thus made the same in each instance. A dose of 5 minims of thyroid residue which contained

8.2 mgs. of protein was taken as the base or standard. This, when mixed with the 275 cc. of Locke's solution in the cylinder holding the unstriated muscle segments, amounted to a dilution of approximately 1 in 10,000.

Effects upon the contractions of the cat's uterus
(Average results from many experiments.)

SUBSTANCE TESTED	REACTION	SYMBOL
Thyroid residue.....	Very strong	(++++)
Alcohol soluble part.....	Perceptible	(+)
Alcohol insoluble part.....	Doubtful	(±)
Thyroid globulin.....	None	(0)
Thyroid coagulated proteins.....	None	(0)
Thyroid nucleo-proteins.....	Doubtful	(±)
Thymus residue.....	Perceptible	(+)
Thymus globulin.....	None	(0)
Thymus nucleo-protein.....	None	(0)
Spleen residue.....	Good	(++)
Spleen globulin.....	None	(0)
Pituitary residue.....	Very strong	(++++)
Pituitrin (Burroughs Wellcome).....	Very strong	(++++)
Pancreas residue.....	Perceptible	(+)
Pancreas nucleo-protein.....	Doubtful	(±)
Liver residue.....	Good	(++)
Alcohol soluble part.....	Perceptible	(+)
Liver nucleo-protein.....	Doubtful	(±)
Parathyroid residue.....	Very strong	(++++)
Adrenal residue.....	Perceptible	(+)
Adrenal nucleo-protein.....	None	(0)
Adrenal globulin.....	None	(0)

Of the large number of tracings required to establish these findings, there are submitted for inspection only two which illustrate the average of the results in the cases of the residues of the thyroid (Fig. 1), and pituitary glands (Fig. 2). The addition of a few drops of 1:1000 adrenalin chloride solution causes an immediate paralysis of the previously induced and more or less characteristic residue contractions. This paralyzing effect of epinephrin upon the uterus is well recognized. The adrenal residue contains much epinephrin but does not like adrenalin paralyze the contractions induced by other residues, except in the case of the pituitary residue. The characteristic contractions induced by the pituitary residue are paralyzed by the adrenal residue as they are also by adrenalin chloride.

In all of these experiments contractions were produced mainly by the residue portion of the extracts. Those which resulted from the addition to the Locke's solution of thyroid or of parathyroid residues

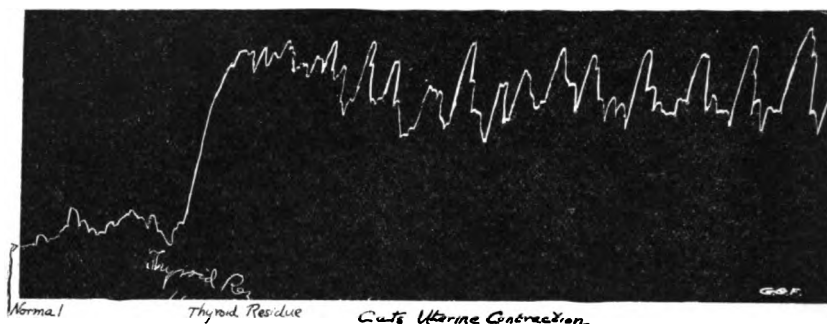


Fig. 1. Thyroid residue. Cat's uterine contraction.

were of longer duration than those from the pituitary residue. With the parathyroid residue the muscle reaction consists of regular, vigorous contractions followed by partial relaxations. The contractions are at first of the clonic type, but soon pass into the tonic type. The ef-

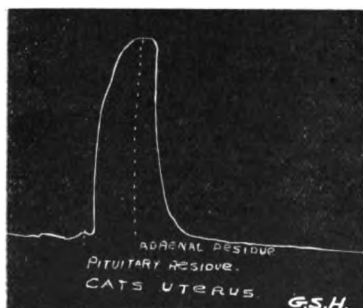


Fig. 2. Effect of pituitary residue on cat's uterus followed by adrenal residue.

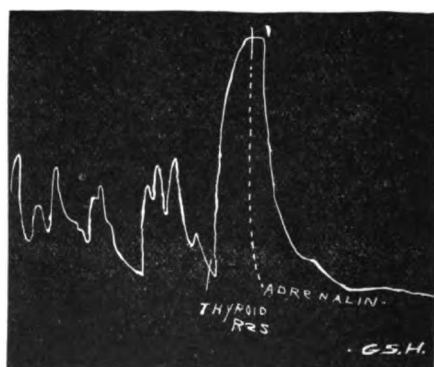


Fig. 3. Effect of thyroid residue on cat's ileum followed by adrenalin chloride.

fects of the thyroid residue are about as pronounced as those from the parathyroid residue, but differ in that fibrillary twitchings are superimposed on the vigorous contractions, and later there is a less marked type of tonic contractions.

With the same method and the same dosage we next tested the effects of the different fractions of aqueous extracts of organs upon the contractions of the muscle segments taken from the duodenum, ileum and the proximal and distal portions of the colon.

The results have been recorded in the following table:

7

SUBSTANCE TESTED	DUODENUM		REACTION UPON MUSCLE FORM				DISTAL PT.	
			Ileum		Colon Proximal pt.			
Thyroid residue	Strong	(+++)	Very strong	(++++)	Perceptible	(+)	None	(0)
Alcohol soluble part	Strong	(+++)	Perceptible	(+)	None	(0)	None	(0)
Alcohol insoluble part	Perceptible	(+)	None	(0)	None	(0)	None	(0)
Thyroid globulin	None	(0)	None	(0)	None	(0)	None	(0)
Thyroid nucleo-protein	Perceptible	(+)	Perceptible	(+)	None	(0)	None	(0)
Thyroid coagulated protein	None	(0)	None	(0)	None	(0)	None	(0)
Parathyroid residue	Very strong	(++++)	Very strong	(++++)	Good	(++)	Strong	(+++)
Liver residue	Perceptible	(+)	Good	(++)	None	(0)	Perceptible	(+)
Alcohol soluble part	Perceptible	(+)	Strong	(+++)	Perceptible	(+)	Good	(++)
Liver nucleo-protein	None	(0)	None	(0)	None	(0)	None	(0)
Spleen residue	Doubtful	(±)	Perceptible	(+)	Perceptible	(+)	Doubtful	(±)
Spleen nucleo-protein	None	(0)	None	(0)	None	(0)	None	(0)
Spleen globulin	None	(0)	None	(0)	None	(0)	None	(0)
Thymus residue	Doubtful	(±)	Very strong	(++++)	Perceptible	(+)	Doubtful	(±)
Thymus globulin	None	(0)	Doubtful	(±)	None	(0)	None	(0)
Thymus nucleo-protein	None	(0)	Perceptible	(+)	None	(0)	None	(0)
Pancreas residue	Perceptible	(+)	Strong	(+++)	Perceptible	(+)	Perceptible	(+)
Pancreas nucleo-protein	None	(0)	None	(0)	None	(0)	None	(0)
Adrenal residue	Perceptible	(+)	Good	(++)	Perceptible	(+)	Perceptible	(+)
Pituitary residue	Strong	(+++)	Strong	(+++)	Strong	(+++)	Strong	(+++)

After the contraction induced by any residue has become manifest, the addition of adrenalin produces an immediate relaxation. This is shown in Figs. 3, 4 and 5.

From these findings it appears that the globulins and coagulable proteins of all organs show no effect upon the contractions of smooth muscle fiber from any part of the gut. The nucleo-proteins rarely gave a perceptible or doubtful reaction which might indicate a content of some proenzyme or incompletely formed active principle.

The thyroid residue does not act on all parts of the intestine with equal force. It produces a strong reaction upon the duodenum and a very strong reaction upon the ileum. But upon the proximal portion of the colon its reaction is slight and upon the distal part none.

The parathyroid residue produces a very strong reaction upon the duodenum and ileum, but only a good reaction upon the proximal part of the colon, while upon the distal portion its reaction is very strong.

The pituitary residue seems to produce the same vigorous reaction upon all parts of the small and large intestine.

The thyroid, parathyroid and pituitary all have a similar pronounced effect upon both uterine and intestinal muscle. The pancreas produces a strong reaction upon the ileum, but only a perceptible reaction upon the remainder of the intestine and the uterus.

The adrenal residue shows effects which are very similar to those of the pancreas.

The thymus residue produced a very strong reaction upon the ileum, but only a very slight or doubtful reaction upon the colon and the duodenum. The residues of the spleen, and liver were only mild stimulants to intestinal contractions.

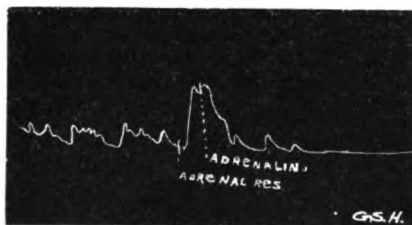


Fig. 4. Effect of adrenal residue on cat's ileum followed by adrenalin chloride.

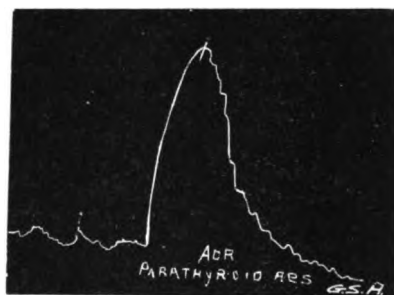


Fig. 5. Effect of parathyroid residue on cat's ileum followed by adrenalin chloride.

CONCLUSIONS.

(The term "residue" refers to that portion of an aqueous extract of an organ which remains after the removal of the nucleo-proteins, globulins and coagulable proteins.)

1. The residues of an aqueous extract of the pituitary, pineal, thyroid, parathyroid, thymus and adrenal glands, and from the liver, pancreas and spleen, contain most, if not all, of the internal secretions of these organs.

2. Each residue produces a characteristic stimulating effect upon the unstriated muscle fibers of the cat's uterus. This stimulation is paralyzed by adrenalin.

3. The residue of each organ acts differently upon different portions of the unstriated muscle fibers of the cat's intestine. When the residue

stimulates the contraction, the addition of adrenalin produces an immediate paralysis.

4. Adrenalin is generally accepted as acting upon the intermediate substance between the end plates of the terminal filaments of the sympathetic and unstriated muscle. Therefore, these residues of organs must act upon some portion of the termination of the sympathetic nerves, and each residue produces its effect by a different chemical or physico-chemical action.

THE FEEDING OF YOUNG CHICKS ON GRAIN MIXTURES OF HIGH AND LOW LYSINE CONTENT

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It has been shown by Osborne and Mendel (1) in an exhaustive series of experiments on the feeding of albino rats, that lysine is primarily responsible for the stimulation of growth. In this connection E. H. Nollau (2) and, independently, Grindley, Joseph and Slater (3) have recently made quantitative determinations of the amino-acids contained in various commercial feeding stuffs and other sources of protein by the Van Slyke (4) method, with the view of determining the lysine content of such mixtures. In this connection, it also occurred to one of us, Kastle, that it would be of interest to conduct a series of feeding experiments on young chicks, using grain mixtures containing a low and high lysine content respectively. With this end in view, two lots of young chicks of mixed breed, each lot containing ten chicks and selected entirely at random from the entire lot, were chosen for the experiment. These lots are herein designated as Lot I and Lot II, respectively. During this feeding experiment, which lasted over a period of eight weeks, viz, from May 13 to July 6, 1915, these chicks were kept under strictly comparable conditions, so far as their general habits of life were concerned. The experiments were carried on in a basement room of the Experiment Station building, floored with concrete, the temperature of which ranged from 23.5°C. to 27°C., and which during a part of the day was exposed to sunlight from basement windows having a southeasterly exposure. The two lots of chicks were separately confined in wire runs, 4½ by 4 feet, the bottom of the run being floored with common lumber and covered over with a layer of earth and straw. All of the chicks were freely supplied with water, gravel, charcoal and oyster shells during the progress of the experiment and

¹ The feeding experiments described in this paper were carried on entirely by Messrs. Buckner and Nollau.

about once a week there was placed in each run a piece of grass sod from one to two feet square. All of the chicks were supplied at intervals with sprouted oats, and each run was provided with a hover of the usual type in which the chicks were placed at night.

At the beginning of the experiment the weights of the two lots of chicks were as follows: Lot I (10 chicks) 438 grams, and Lot II (9 chicks) 338 grams, or an average weight per chick, in Lot I, of 43.8 grams, and in Lot II, of 43.1 grams. The chicks of Lot I received a mash twice a day, morning and evening, consisting of equal parts by weight of finely ground wheat, wheat bran, sunflower seed and hemp seed, moistened with skimmed milk, and once a day at noon, they were given a coarsely ground grain mixture of wheat, hemp seed and cracked corn.

On hydrolysis the mash fed to the chicks of Lot I gave the following numbers for amino-acids, by the Van Slyke method:

Ammonia N.....	12.09
Melanin N.....	7.42
Cystine N.....	2.09
Arginine N.....	17.48
Histidine N.....	3.28
Lysine N.....	3.80
Amino N filtrate.....	38.53
Non-amino N filtrate.....	13.94
Total.....	98.63

Towards the end of the experiment the sprouted oats furnished these chicks was replaced by cabbage. The mash, exclusive of the milk, contained 18.09 per cent of protein, and the grain mixture 16.29 per cent protein.

The grain mixture fed to Lot I gave, on hydrolysis, the following numbers for amino-acids:

Ammonia N.....	11.66
Melanin N.....	10.50
Cystine N.....	2.72
Arginine N.....	11.36
Histidine N.....	6.21
Lysine N.....	2.23
Amino N filtrate.....	41.89
Non-amino N filtrate.....	15.67
Total.....	102.24

The mash was prepared with a small amount of sour skimmed milk varying between 15 and 20 cc. In this connection it should be borne in mind that owing to the habits of the chicken it is practically impossible to feed any given lot of chickens precisely the same amount of food at any one time, or rather, it is impossible to determine, except in a general way, whether they have eaten all the food presented to them at any one time. This difficulty was overcome as completely as possible in this work by feeding each lot of chickens approximately the amount of food that they would clean up at any particular time, these several amounts being accurately weighed at every feeding. For example, in the morning, at eight o'clock, Lot I received 30 grams of the mash, and at noon they received from 15 to 25 grams of the grain mixture. Then at 5 p.m. they received a second 30-gram portion of the mash. In the same way Lot II received 30 grams of a mash consisting of finely ground barley, rice, hominy and oats, 100 grams each, and 56 grams of gluten flour. This mash contained 18.1 per cent of protein, and gave the following numbers for amino-acids on hydrolysis:

Ammonia N.....	17.85
Melanin N.....	7.22
Cystine N.....	4.29
Arginine N.....	5.93
Histidine N.....	7.38
Lysine N.....	.50
Amino N filtrate.....	42.99
Non-amino N filtrate.....	15.68
Total.....	101.84

At noon the chicks of Lot II received from 30 to 50 grams of a grain mixture consisting of equal parts of barley, rice and hominy, which gave the following numbers for amino-acids on hydrolysis:

Ammonia N.....	12.68
Melanin N.....	9.40
Cystine N.....	1.95
Arginine N.....	6.77
Histidine N.....	7.76
Lysine N.....	.79
Amino N filtrate.....	40.08
Non-amino N filtrate.....	20.25
Total.....	99.68

This grain mixture was found to contain 8.74 per cent of protein and hence twice as much of it was fed to the chicks of Lot II as of the grain

mixture fed to Lot I. The mash supplied to the chicks of Lot II was prepared with protein-free milk.² The two lots of chickens were weighed at the beginning of the feeding experiments, at noon on May 13, and again at intervals shown in the following tables:

TABLE I—LOT I

DATE	NO. OF CHICKS	TOTAL WEIGHT OF CHICKS	AVERAGE WEIGHT OF EACH CHICK	AVERAGE INCREASE IN WEIGHT IN GRAMS	PERCENTAGE AVERAGE INCREASE
1915					
May 13.....	10	438	43.8		
May 19.....	9	486	54.0	10.2	23.30
May 25.....	7	540	77.1	23.1	42.77
June 1.....	7	802	114.6	37.1	47.82
June 8.....	7	1113	159.0	44.4	38.74
June 15.....	7	1517	216.7	57.7	36.30
June 23.....	5	1650	330.0	113.3	52.20
June 29.....	5	2063	412.6	82.6	25.00
July 6.....	5	2553	510.6	98.0	23.75

TABLE II—LOT II

DATE	NO. OF CHICKS	TOTAL WEIGHT OF CHICKS	AVERAGE WEIGHT OF EACH CHICK	AVERAGE INCREASE IN WEIGHT IN GRAMS	PERCENTAGE AVERAGE INCREASE
1915					
May 13.....	9	388	43.1		
May 19.....	9	450	50.0	6.9	16.00
May 25.....	8	477	59.6	9.6	19.20
June 1.....	8	588	73.5	13.9	23.32
June 8.....	8	707	88.3	14.8	20.10
June 15.....	8	848	106.0	17.7	20.00
June 23.....	7	928	132.5	26.5	25.00
June 29.....	7	1086	155.1	22.6	17.00
July 6.....	7	1195	170.7	15.6	10.00

It will be seen from the above results that 5 chickens of Lot I at the conclusion of the experiment weighed 2553 grams, whereas 7 chickens

² The protein-free milk used in these experiments was prepared according to the method described by Osborne and Mendel (Carnegie Pub.) except that the casein was precipitated with lactic acid and the product neutralized with lime, our reason for making this change in the original directions of Osborne and Mendel being to eliminate common salt from the ration, which is said to be toxic to the chicken.

of Lot II weighed 1195 grams, or 5 average chickens of Lot II would have weighed 853.5 grams. The striking difference in these two lots of chicks is further shown by a comparison of the photographs of the two lots made on July 5. In this connection it should be borne in mind that the focal distance was the same in both instances so that the photographic comparisons are exact. One of the photographs shows the largest and best developed cockerel of each of the two lots. It will be observed that there are marked differences in the feathering of the two lots of chickens, Lot I showing the feathering characteristic of the mature chicken, whereas Lot II still showed the feathering of the young and immature chick at the conclusion of the experiment. Great difference in the two lots of chickens was also shown in their general activity during the progress of the experiment, the chickens of Lot I being greatly more active than the chickens of Lot II. It was also observed that the chickens of Lot II consumed more charcoal than the chickens of Lot I. No diarrhea or other evidences of digestive disturbances were observed except in the case of one chicken of Lot I and this was only noticeable one day. Five chickens of Lot I and three of Lot II died during the early progress of the experiment, one of the chicks of Lot II dying before the feeding experiment was actually begun.³ During the progress of the experiment both lots of chickens were abundantly supplied with tap water to which a small amount of lime was added each day.

It is well known that certain of the materials used in the make up of the ration of Lot I have been used to advantage in feeding fowls and birds. For example, the value of sunflower seed and skimmed milk as a feed for chickens is well recognized by poultrymen and hemp seed always forms a large part of the ration of small birds in captivity, such as the canary. The desire shown by the young chick for hemp seed is remarkable. It has been observed that out of a grain mixture containing this material, they will pick out every hemp seed before eating the remainder of the ration, and in this connection it is of interest to note in passing that of all of the substances used in our feeding experiments, hemp seed is richest in lysine.

³ The high mortality shown by these two lots of chicks is inexplicable for the reason that they received all possible attention and were kept under sanitary surroundings. In this connection it should be borne in mind, however, that chickens hatched late in the spring show an abnormally high mortality as compared with chickens hatched very early in the spring. This is probably due to loss of virility on the part of the cock fertilizing the eggs.

At the conclusion of the feeding period on July 6, the chickens of Lot II were put on the rations fed to Lot I. On July 13, the chickens of Lot II were found to weigh 1539 grams, showing a total gain of 344 grams for the lot, or an average weight per chick of 219.9 grams, as compared with 170.7 grams per chick on July 6, or an increase in 7 days of 49.2 grams per chick as compared with an average gain per week of 15.9 grams during the regular period of the experiment.⁴

The chicks used in these experiments were hatched under the hen on May 9. They were brought to the Experiment Station on the 11th and the feeding experiment was begun on May 13. In the interval between May 11 and May 13 all of the chickens received small amounts of Purina chick feed containing 11 per cent protein.

It is evident from these results that whereas the chickens of Lot I grew normally, the chickens of Lot II were undoubtedly stunted in their growth. This difference in the nutrition of these two lots of chicks is due, in all probability, to the difference in the amount of lysine received by the two lots and possibly to a difference in the quantity and nature of the fats contained in the two rations. Thus the mash fed to the chicks of Lot I contained 13.08 per cent of fat, and the dry grain mixture 8.21 per cent, whereas the mash fed to the chicks of Lot II contained only 1.8 per cent of fat, and the grain mixture 1.0 per cent. It should be borne in mind in this connection, that Osborne and Mendel (5) and also McCollum (6) and his associates have shown that certain of the natural fats contain substances which stimulate animal growth. Such fats are known to be present in butter, cod liver oil, the yolk of the egg, corn meal (7), etc., and such growth-promoting fats are doubtless contained in many other grains and vegetable prod-

⁴ This, in itself, is a striking confirmation of the fact that the ration fed to the chickens of Lot I is greatly in excess of the ration fed originally to the chicks of Lot II in its power to stimulate growth. This rapid increase in weight shown by the chicks of Lot II during the time that they were on the ration of Lot I, indicates that while their growth was stunted on the first ration, they still possessed the power to grow rapidly on the ration of Lot I. In this connection it may be said that during the long period in which the chicks of Lot II were on the original ration, they were not only stunted in growth but very timid, especially for hand raised chicks, whereas during the shorter period of one week, during which time they were on the ration of Lot I, they became much less nervous and excitable and very gentle. Another striking difference between the chicks of Lots I and II is that shown in the combs of the cockerels of the two lots, the combs of the cockerels of Lot I being red and well developed, whereas the combs of the cockerels of Lot II were pale and undeveloped. This is clearly shown in photograph 5.

ucts. Such being the case we would be unwarranted in attributing the greatly increased growth of the chicks of Lot I as compared with the chicks of Lot II, entirely to differences in the character of the proteins furnished the two lots of chicks. The difference shown by the two lots, however, is striking and unmistakable. It, therefore, remains to be determined whether this difference is due to differences in the protein or fats, one or both.

In order, therefore, to throw further light upon this point, two lots of pure bred white Leghorn chicks, Nos. III and IV, were fed upon the same rations that were fed to Lots I and II except that to the ration supplied to Lot IV there was added sufficient butter fat to bring the fat content up to that of the ration fed to Lot I; our reason for using butter fat in this connection being that McCollum and Mendel have shown this to be a growth-promoting fat.⁵

The feeding experiments on Lots III and IV were carried out in the following manner: out of the same lot of pure bred white Leghorn chicks, twelve chicks were selected at random from the entire lot to compose Lot III, the remaining twelve composing Lot IV. Lot III was found to weigh 488 grams or an average of 40.6 grams per chick. Lot IV was found to weigh 471 grams, or an average of 39.25 grams per chick.

Lot III was fed on mash No. 1 morning and evening, and on grain mixture No. 1 at noon. Lot IV was fed on mash No. 2 morning and evening and on grain mixture No. 2 at noon, the mash No. 2 and grain mixture No. 2 containing butter fat. Both of these lots received shredded cabbage, which they ate with avidity, and in both runs a square foot of fresh sod was placed every other day. The chicks of both lots also had free access to charcoal, gravel, oyster shells and water to which a small amount of lime was added daily and in which at intervals was placed a small amount of permanganate.

The results of these feeding experiments are given in Tables III and IV.

It will be seen from Table III that five of the chicks died during the first three weeks of the feeding period.

⁵ Obviously the best method of settling this point would have been to add to the ration fed to the chicks of Lot II the fat extracted from the ration fed to Lot I in the quantities contained in the ration of Lot I, viz.: 13.08 per cent in the mash and 8.21 per cent in the grain mixture. Experimental difficulties were encountered, however, in the effort to extract the fat contained in the rations fed to Lot I.

TABLE III—LOT III

DATE	NO. OF CHICKS	TOTAL WEIGHT OF CHICKS	AVERAGE WEIGHT OF CHICKS	AVERAGE INCREASE IN GRAMS	AVERAGE PERCENT-AGE INCREASE	GRAMS FEED PER CHICK PER DAY
1915						
July 1.....	12	488	40.6			5.3
July 9.....	9	487	53.8	13.2	32.5	10.0
July 15.....	9	607	67.4	13.64	25.3	10.0
July 22.....	7	625	89.4	22.0	32.5	12.8
July 29.....	7	945	135.0	45.6	51.0	15.0
August 5.....	7	1315	185.0	50.0	37.0	18.0
August 12.....	7	1657	236.7	51.7	27.9	21.0
August 19.....	7	2000	285.7	49.0	20.7	24.0
August 26.....	7	2510	358.5	72.8	25.4	27.0
August 29.....	7	2769	395.6	36.8	10.3	27.0
September 4.....	7	2886	412.3	17.0	4.3	27.0
September 11..	7	2988	426.8	14.5	3.5	27.0
September 18.....	7	3200	457.1	32.3	7.5	27.0

TABLE IV—LOT IV

DATE	NO. OF CHICKS	TOTAL WEIGHT OF CHICKS	AVERAGE WEIGHT OF CHICKS	AVERAGE INCREASE IN GRAMS	AVERAGE PERCENT-AGE INCREASE	GRAMS FEED PER CHICK PER DAY
1915						
July 1.....	12	471	39.25			5.3
July 9.....	10	461	46.1	6.85	17.4	10.0
July 15.....	10	517	51.7	5.6	12.1	10.0
July 22.....	6	322	54.5	2.8	5.4	12.8
July 29.....	6	398	66.3	11.8	21.65	15.0
August 5.....	6	462	77.0	10.7	16.1	18.0
August 12.....	6	515	85.8	8.8	11.4	21.0
August 19.....	6	592	98.6	12.8	14.9	24.0
August 26.....	6	660	110.0	11.4	11.5	27.0
August 29.....	6	708	118.0	8.0	7.3	27.0
September 4.....	5	863	144.0	26.0	22.1	27.0
September 11.....	5	931	186.2	42.2	29.3	27.0
September 18.....	5	1178	235.6	41.4	26.5	27.0

At the beginning of this feeding experiment, July 1, seven average chicks of Lot III weighed 284.2 grams or an average of 40.6 grams. At the end of the feeding experiment, on August 29, the 7 chickens weighed 2769 grams, or an average of 395.6 grams per chick, or within a period of 59 days each chick, of Lot III gave an average gain of 355.

grams. On the other hand it will be seen from Table IV that 6 chicks of the lot died within the first three weeks of the feeding experiment. At the beginning of the feeding experiment, six average chicks of this lot weighed 235.5 grams, or an average of 39.25 grams per chick. At the end of the feeding period, on August 29, these 6 chicks weighed 708 grams, or an average of 118 grams per chick. In other words the chicks of Lot III showed an average gain per chick of 277.6 grams over the chicks of Lot IV or a percentage gain of 235.

The weekly gains of these two lots of chicks are shown in Tables III and IV. The differences shown by these two lots of chicks, at the end of the feeding period, were very striking. The chickens of Lot III were strong, growthy and perfectly feathered, in contrast to the chicks of Lot IV which although in perfect health, were markedly stunted in their growth and showed the feathering characteristic of a much younger chick. All of the chicks of Lot IV, for example, at the end of the feeding period, still showed the yellow color and appearance of the newly hatched chick about the head and neck. The external sexual characteristics of these two lots of chicks showed also the most striking differences. In Lot III the cockerels, for example, were easily distinguished from the hens and both showed well developed, highly colored gills and combs, whereas the chicks of Lot IV showed no well developed external sexual characteristics whatever, it being impossible to distinguish between the cockerels and the hens, the combs of both being rudimentary and colorless. These differences are shown to some extent in the photographs (8 to 10 inclusive) of these two lots of chickens, which were made on August 27. On the other hand, nothing except a colored photograph could bring out fully the striking differences shown by these two lots of chicks.

On August 29 the rations fed to these two lots of chicks were reversed, with the most striking result, as also shown in Tables III and IV, the numbers being given in *italics*. It will be seen that the average percentage gains of Lot III are 5.1 against 25.9 for Lot IV for the three weeks. Within one week after reversing the rations fed to Lots III and IV the external sexual characteristics of the chicks of Lot IV became noticeable and at the end of three weeks were very pronounced. The yellowish down about the heads of the chicks of Lot IV had disappeared and the feathering is becoming that of the normal fowl of this age.

It is evident from these results that the marked differences shown by these two lots of chicks in rate of growth and development cannot



95. COCKERELS FROM LOT I. AND LOT II.



10. SIDE VIEW OF THE TWO
HENS FROM LOTS III AND IV.

In the legends, wherever the words "another
new" occur, it signifies that several photographs
were made of these two lots of chicks. To save
space, however, it has been found necessary to
reduce the number of illustrations and hence
these particular photographs have been selected.

PHOT
A

be ascribed to the fat content of the two rations, but rather to differences in the amino-acid content of the two rations and in all probability to differences in the lysine content.

As indicated in the above, we have not as yet been able to satisfactorily extract the fats and oils contained in mash and grain mixtures No. 1. We are still hopeful of accomplishing this, however, in order to determine the effect of adding the fats and oils contained in ration No. 1 to ration No. 2.

In conclusion, we desire to point out the value of feeding experiments with the chicken both from a practical and scientific standpoint. The financial returns to poultrymen from a successful method of feeding young chicks and laying hens would be enormous. From a purely scientific standpoint, the young chick lends itself well to feeding studies, for the reason that it reaches maturity in from nine months to one year, depending on the breed. For these reasons, it is proposed to continue these investigations as rapidly as time will permit.

- (1) OSBORNE AND MENDEL: *Journ. Biol. Chem.*, 1914, xvii, 325; *Zeit. f. Physiol. Chem.*, 1912, lxxx, 307; *Carnegie Inst. of Washington, Pub. No. 156*, pts. 1 and 2.
- (2) NOLLAU: *Jour. Biol. Chem.*, 1915, xxi, 611.
- (3) GRINDLEY, JOSEPH AND SLATER: *Jour. Amer. Chem. Soc.*, 1915, xxxvii, 1778.
- (4) VAN SLYKE: *Jour. Biol. Chem.*, 1911, x, 15.
- (5) OSBORNE AND MENDEL: *Jour. Biol. Chem.*, 1913, xvi, 423; 1914, xvii, 401.
- (6) MCCOLLUM: *Jour. Biol. Chem.*, 1913, xv, 167.
- (7) MCCOLLUM: *Jour. Biol. Chem.*, 1915, xxi, 179.

ELECTRICAL STUDIES IN MAMMALIAN REFLEXES

II. THE CORRELATION BETWEEN STRENGTH OF STIMULI AND THE DIRECT AND REFLEX NERVE RESPONSE

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INTRODUCTION

In a preceding paper¹ we have described the flexion-reflex in the decerebrate cat as recorded electrically in a flexor muscle and its motor nerve. In the present paper we propose to report certain observations on the gradation in intensity of the stimuli employed during these experiments, and to discuss the bearing of these observations on the spread of reflexes and the doctrine of "graded synaptic resistance." We have observed the reflex responses to a series of single induction

¹ Forbes and Gregg: This Journal, 1915, xxxvii, 118.

shocks graded as to intensity, and subsequently recorded from the afferent nerve used to induce the reflex, monophasic action currents corresponding to a similar series of stimuli applied in the same way.

The subject of the correlation of the intensity of muscular response with the intensity of reflex stimulus has been summarized by Sherrington.² He states that the "correspondence between the intensity of external stimulus and reflex end-effect" is less close than that found in comparing the stimulus applied to a motor nerve with either the contraction of the innervated muscle or the electrical response of the nerve itself. He cites Biedermann as finding an "all-or-none" relation in the reflex response of a cooled frog to single induction shocks. But he shows clearly that "graded intensity of reflex-effect does occur." He demonstrates this fact specifically in the spinal mammal in the case of the flexion-reflex (which has been the subject of our experiments), the scratch-reflex and the crossed extension reflex; although he finds an approximately "all-or-none" relation in the "extensor-thrust." In the gradation of response prolongation is even more marked than increase in intensity. It should be noted that in all Sherrington's cases the stimuli were not single but repeated, and applied to the skin and not to an afferent nerve trunk. The importance of these facts will be considered later.

In a recent paper,³ published since the completion of our experiments. Sherrington and Sowton report that with single induction shocks of graded intensity applied to an afferent nerve trunk, a more extensive gradation of flexor reflex contraction is found than in the case of the same muscle stimulated directly through its motor nerve. They show that grading in the reflex persists through a range of stimuli subsequently shown to be supramaximal for the nerve-muscle preparation. Their records are all myographic and they furnish no direct evidence as to gradation of activity in the stimulated afferent nerve trunk itself.

In regard to the spread of reflexes resulting from an increase in the intensity of stimulation, Sherrington wrote as follows:⁴ "If by appropriate stimulation of the skin of the foot . . . the ordinary flexion-reflex of the hind limb of the dog be evoked, the extent of the reflex increases with increase in the intensity of the stimulus. The re-

² Sherrington: *Integrative Action of the Nervous System*, 1906, 70-72.

³ Sherrington and Sowton: *Journ. Physiol.*, 1915, xlix, 331. Cf. also Graham Brown: *Proc. Roy. Soc. B.*, lxxxvii, 132. The latter showed extensive grading of reflex contraction in tenuissimus muscle.

⁴ Sherrington: *Op. cit.*, 150.

flex-effect spreads over a larger and larger field, irradiating as it were in various directions from a focus of reflex-discharge which takes effect on the limb itself."

Before describing the results of our experiments, it will be well to summarize the method which is described in full in our preceding paper, and to describe such additional procedures as were employed for the purpose at hand.

The flexion-reflex in the decerebrate cat has been examined with the string galvanometer by the following method. Decerebration under profound ether anaesthesia has been performed in the usual way.⁵ The essential features of the operation are the ligation of both carotid arteries and the removal of the entire cerebrum above the level of the posterior corpora quadregemina. A preparation is thus obtained which remains in decerebrate rigidity without manifesting other muscular action unless stimulated. For stimulation a pair of Sherrington shielded electrodes was applied to the popliteal nerve cut distal to the point of stimulation. These were connected with the secondary coil of a Berne inductorium in such a way that break shocks should be ascending (i.e., with cathode central). The coil (designated coil *F*) which is 14 cm. long, was calibrated in accordance with Martin's method,⁶ and the current in the primary coil was measured with an ammeter. The flexion-reflex was thus elicited by means of single induction shocks of known value applied directly to an afferent nerve. The action current of the motor nerve (peroneal) supplying the tibialis anticus muscle in the same leg was led off with a pair of non-polarizable "boot" electrodes and recorded photographically from the string galvanometer. In most cases the nerve was crushed between the leading-off electrodes to render the action currents monophasic. In almost all cases the peroneal nerve was subsequently severed at the hip and stimulated directly just distal to the point of severance while the leading-off electrodes remained in position, in order to obtain for comparison records of the action currents under direct stimulation.

Most of these experiments also supplied the results to be reported in the present paper. The principal procedures employed for the purposes of the latter and not already described were the variation over a wide range of the strength of stimulus used to evoke the reflex and the subsequent recording of monophasic responses directly from the

⁵ See Forbes and Sherrington: *This Journal*, 1914, xxxv, 367.

⁶ Martin: *Measurement of Induction Shocks*, New York, 1912, 55.

afferent nerve with a similar series of stimuli. Further details of procedure will be described as the need arises.

In describing the results it will be well to begin with the action currents led off from the afferent nerve in response to induction shocks of graded intensity. Few experiments seem to have been reported dealing with the gradation of electrical responses in nerve trunks. Waller in 1895⁷ applied tetanizing stimuli to a frog's sciatic nerve for periods of one-eighth minute each and recorded simultaneously the mechanical lift of the innervated muscle and the "negative variation" of the demarcation current at the central end of the nerve. The negative variation was recorded with a slow moving galvanometer which showed by a single excursion the whole series of electrical responses resulting from the continued tetanization. He reports that on grading the intensity of stimuli in a series of such observations the negative variation increases in proportion to the stimulus even after maximal contraction is obtained. His statement is: "On nerve the curve expressing the relation between cause and effect is a straight line, the effect is proportional to the cause, at least within moderate ranges of excitation, exceeding, however, the upper limit of maximum functional effect, as gauged by muscular contraction." He does not appear, however, to have extended his observations to stimuli much more than double the threshold value.

Lucas⁸ more recently showed evidence which favored the view that in a single skeletal muscle fiber, excited through its motor nerve, the contraction follows the "all-or-none" law. But in commenting on his results he pointed out that this conclusion "does not imply that the nervous impulse is always maximal any more than it implies that the electric current used to excite the nerve is always maximal." He further remarks that the observation of Waller mentioned above does not, as might at first appear, prove gradation of response in the individual nerve fiber, since muscular contraction was in this case recorded in the gastrocnemius, and there are many fibers in the sciatic nerve which do not innervate this muscle. These might have been responsible for the increment in the negative variation after the muscular contractions had become maximal. It should also be noted that in Waller's experiment the leading-off electrodes were at the central end of a frog's sciatic nerve and the stimulating electrodes were placed midway be-

⁷ Waller: *Brain*, 1895, xviii, 208.

⁸ Lucas: *Journ. Physiol.*, 1909, xxxviii, 132.

tween these and the muscle. Thus the stimulating electrodes cannot have been more than 3 or 4 cm. at most from the leading-off electrodes. We find evidence, to be described presently, indicating that with strong induction shocks electrical disturbances may appear in the recording instrument which seem to be distinct from the action current; and at such close proximity to the stimulating electrodes their effects may be considerable. A slow moving galvanometer could not have differentiated between different kinds of electrical effect. It seems, then, that Waller's experiment does not prove the possibility of gradation of electrical response in a single nerve fiber, nor does it show that the electrical response of a whole nerve trunk increases without limit in response to increase in the strength of the stimulus. Lucas concluded his discussion of the subject with the following statement: "We must therefore regard the question whether the response of a nerve fiber is capable of gradation as being at present undecided."

Gotch⁹ in 1902 studied the electrical response in nerve to a single induction shock with a capillary electrometer. His chief concern was the comparison of the time relations in maximal and submaximal responses. He gave no detailed statement concerning the gradation of magnitude in the responses. He estimated the strength of shock requisite for minimal and maximal stimulation by means of the resulting muscular contraction. In order to obtain both submaximal and maximal responses he made his experiments "with intensities of induction shocks starting slightly weaker than the above minimal value and increasing to beyond that of the maximal one;" how far beyond the maximal value is not stated, but apparently the entire range of intensities was not great. The only statement in Gotch's paper in regard to the gradation of magnitude in the electrical response is the following:¹⁰

" . . . it seems clear that the electrical response of nerve to a single stimulus varies as to its magnitude in correspondence with variations in the exciting efficiency of the stimulus, and that, like muscle, the response of nerve passes through a series of submaximal stages of increasing value until a maximum is reached." So far as we are aware the fact here stated, that a limiting maximal value of the electrical response in nerve is reached as the stimuli are increased in strength, has not been confirmed by other observers, nor did Gotch place more than passing stress on the fact or record accurate measurements with stimuli of widely varying intensity.

⁹ Gotch: *Journ. Physiol.*, 1902, xxviii, 395.

¹⁰ *Loc. cit.*: 405.

Gotch found the time relations in maximal and submaximal responses to be identical, and inferred that such gradation of magnitude as occurred signified chiefly if not wholly a gradation in the number of fibers excited, and that the individual nerve impulse probably obeys the "all-or-none" law. Quite recently Adrian¹¹ has shown that when a nerve is gradually narcotized, muscular contraction being taken as the index of response, a stage is reached just before conduction ceases altogether at which an "all-or-none" relation of response to stimulus is directly demonstrable. By certain ingenious control experiments he seems to have excluded the possibility of arriving at any other conclusion than that the nerve impulse is unaffected by variation in stimulus, i.e., that it obeys the "all-or-none" law. If this be so, and if the action current be a true index of physiological activity, incapable of varying independently of the nerve impulse it denotes, then it is obvious that a limiting maximal value of the action current must be reached when all the fibers in a nerve trunk are excited.

We can see no other explanation of Adrian's observations than the assumption of the "all-or-none" relation, but in view of the far-reaching consequences of this assumption it seems wise to consider the possibility that some other unthought-of explanation may yet be found when the problem is viewed from a wholly new angle. There is also the possibility which Gotch has suggested¹² that the electrical response is not an infallible criterion of physiological activity; in particular, it is conceivable that quantitative differences in the action current might exist where none existed in the activity as judged by any true functional criterion. For instance, even though the propagated disturbance, as judged by its ability to induce contraction in the innervated muscle or by the distance it can travel through a narcotized portion of the nerve,¹³ cannot be varied by any variation in the stimulus, the electrical response might be capable of gradation.

II. EXPERIMENTAL RESULTS

A. *Correlation between stimulus and action current in the nerve trunk.*

Since we find on record no direct proof that the action current in a nerve trunk cannot increase in magnitude beyond a limiting maximal

¹¹ Adrian: Journ. Physiol., 1914, xlvii, 460.

¹² Gotch and Burch: Journ. Physiol., 1899, xxiv, 422; Gotch: Journ. Physiol., 1902, xxviii, 51.

¹³ Cf. Adrian: Journ. Physiol., 1912, xlv, 393.

value however strong the stimulus, we have deemed it worth while to examine this question with some care. Most of our experiments on this point have been made with the cat's popliteal nerve severed at hip and knee, stimulated at the peripheral end and led off at the central end. The reason for this was that we regularly used this nerve to induce reflex responses, and it was desired to examine with a view to gradation the afferent impulses involved in their production, and to do so as soon as possible after the reflex responses had been recorded and under identical conditions of stimulation. Rather than introduce the delay and probable shift of electrode contacts incidental to transferring the nerve to a moist chamber after removing it from the animal's body, the central end was usually laid across a pair of non-polarizable "boot" electrodes in the open air, the stimulating electrodes were kept in as nearly as possible the same position on the nerve and the observations were made as rapidly as possible in order to minimize the effects of drying and other progressive changes which would invalidate the measurements. Some experiments were made on the peroneal nerve, that portion on the leading-off electrodes being in the moist chamber used for recording reflex responses and already described in our preceding paper. In some experiments the whole sciatic nerve was used and in one it was placed in a moist chamber. In all cases the nerve was dissected out by cutting the surrounding fascia with a sharp knife or scissors, and in all cases it was crushed approximately midway between the leading-off electrodes to render the responses monophasic. The platinum stimulating electrodes were so applied that on the break shock the kathode should be nearest the leading-off electrodes; and for quantitative observations break shocks were always used. In this way the possibility of electrotonic block was avoided. The distances between electrodes were kept constant throughout the course of each experiment, except in specified cases when it was purposely varied.

Accurate comparison of the magnitudes of successive electrical disturbances is rendered difficult by the following progressive changes which tend to alter experimental conditions:*

1. The string galvanometer is gradually heated by the passage of the current used to create the magnetic field. The string has not the same coefficient of expansion as the case in which it is held, and its tension therefore undergoes a progressive change. This, in turn, changes the magnitude of excursion resulting from a given electrical disturbance.

*In addition to the changes enumerated below, the gradual cooling of a nerve on removal from the animal's body should be mentioned.

This source of error can be minimized by frequently readjusting the tension, but the resulting delay serves to increase the error due to the other progressive changes.

2. Progressive drying of the nerve acts in two ways: (a) it increases the total resistance in the secondary circuit, thereby reducing the current produced by a given difference of potential; (b) it eliminates the short circuit provided for the action current by moisture on the surface of the nerve; for the greater the layer of conducting fluid on the nerve, the smaller must be the proportion of the total action current which goes through the string. Of these two factors acting as the nerve dries (a) tends to reduce the excursions of the string, (b) simultaneously tends to increase them.

3. Any injury to the nerve through trauma or drying may introduce a region of impairment where the impulses undergo decrement,¹⁴ and may be abolished altogether in some of the fibers. Such a region of impairment seems to extend for some distance along a nerve from the point where a ligature is applied. Even in a moist chamber we have found the magnitude of the action currents to decrease considerably in less than half an hour if the stimulus was applied near the ligatured end, although the strength of stimulus and all other experimental conditions remained unchanged. We have further found that stimuli applied near where a nerve has been ligatured do not produce nearly so large action currents as when applied far from the seat of injury. Experiment 28 was a striking example of this. The sciatic nerve being ligated and cut at the knee, was stimulated centrally and the reflex responses were noted. An hour after the nerve was ligated it was severed at the hip and crushed as usual in order to record afferent impulses monophasically. It was then placed in a moist chamber; stimulating electrodes were applied about 15 mm. central to the ligature and leading-off electrodes central to these. The nearest lead was about 50 mm. from the point of stimulation. With this arrangement the largest excursions of the string in response to the action current amounted to 4.0 mm.¹⁵ The stimulating electrodes were then shifted about 23

¹⁴ See Adrian: both references cited above; also Lucas: *Journ. Physiol.*, 1913, xlv, 470. Gotch (*Journ. Physiol.*, xxviii, 55) mentions hyper-excitability as occurring in nerve near the seat of a recent injury; this hyper-excitability had probably passed off in our experiments before the observations were begun.

¹⁵ The figures given for excursions of the string refer, of course, to the magnified shadow of the string as appearing on the photographic film. The magnification in all cases was 580 diameters, as stated in our previous paper.

mm. nearer the leads without disturbing the contacts with the latter, and the action currents from maximal stimuli then amounted to about 5.1 mm.

On account of these various sources of error it was difficult to determine with accuracy the gradation of electrical disturbance in response to graded stimuli or to ascertain whether a definite limiting maximal value is reached. Still, by making observations in rapid alternation between two stimuli of different strength, it was possible to nullify almost completely the progressive sources of error, and to obtain valid comparisons. With regard to the effect of stimulating in a damaged portion of the nerve (e.g., close to a ligature), besides such error as is due to the progressive character of the impairment, we should expect additional disturbance of comparisons even with a minimum lapse of time. For, in the first place, the thresholds of those fibers suffering most from injury would probably be much above normal and would differ far more from those of the least injured fibers than in normal nerve; in the second place, it seems probable from the figures just given (Experiment 28) that in such a case some fibers are wholly inexcitable at the point of stimulation, but if strong enough shocks are employed the electrical disturbance will spread along the nerve to a point where the fibers are still excitable. From both these causes it should result that the limiting maximal value, if there be such, is only reached with stronger stimuli than in the case of uninjured nerve, and therefore the gradation of response should be extended over a wider range of stimuli than normal. As will presently appear, our records seem to show just such an increase in gradation when impairment has been present. Consequently, the failure in a given case to find a limiting maximal value below the point where the shocks are so strong that they manifestly introduce non-physiological factors, does not prove that a limiting value is not normally present. On the other hand, any case in which, under favorable conditions, a limiting maximal value is consistently found can be regarded as valid evidence that such a limit to the intensity of electrical disturbance in nerve exists. Any other inference must involve the assumption of some highly improbable coincidence.

We have examined many nerves with the method described, but, for the reasons just given, only a few experiments have afforded grounds for conclusions. In grading electrical responses it must be remembered that the action current in nerve is far too brief to be followed accu-

ately by the string.¹⁶ It is not safe to assume that the excursion of the string is even directly proportional to the maximum difference of potential between the leads; but it is safe to assume that as long as all other conditions remain strictly parallel equal excursions denote equal electromotive forces, and an increase in the excursion denotes an in-

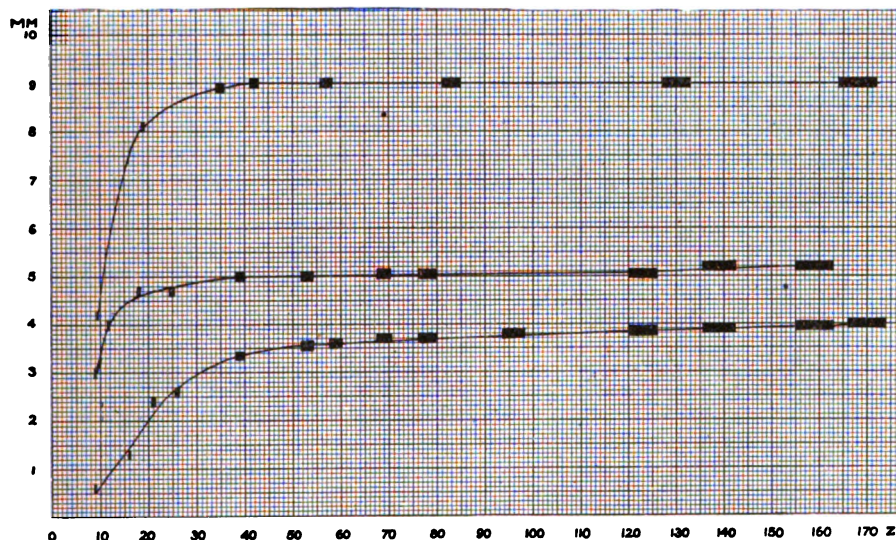


Fig. 1. Relation of monophasic action current to stimulus. Excursion of string in millimeters (ordinates) plotted against Z units (abscissae); only break shocks used. The rectangles mark the limits of experimental and observational error (assuming 2.5 per cent as the possible deviation from the given value of Z).

Uppermost curve: Experiment 21, sciatic nerve.

Lowest curve: first half Experiment 28, sciatic nerve.

Middle curve: second half Experiment 28, same nerve (see text).

The numbers assigned to experiments in this paper correspond to the numbers of the preparations given in our previous paper, the same preparations usually furnishing data for both papers. They are numbered mostly in chronological order, but some early experiments relating only to this paper are numbered out of sequence.

crease in electromotive force. Our chief concern has not been to establish any exact mathematical relationship between stimulus and response, but to determine within what limits gradation of response occurs, i.e., whether a limiting maximal value is reached, and, if so, at about what strength of stimulus. Therefore, the magnitudes of ex-

¹⁶ See Forbes and Gregg: Loc. cit., figure 7, 146.

cursion furnish all the data needed for the desired comparison of electrical disturbances. Thresholds were not accurately recorded since we were concerned chiefly with the approach to maximal stimulation.

The relation between response and stimulus over a wide range is shown in figure 1 (middle curve) taken from Experiment 28, one of the few in which conditions can be considered normal. The measurements are all taken from the second half of the experiment after the stimulating electrodes had been moved to a point 38 mm. from the ligature; the nerve was in a moist chamber and the observations were all made in the course of about twenty minutes. Stimuli of certain strengths were repeated several times, sometimes both before and after weaker or stronger stimuli; in this way approximate constancy of con-

TABLE 1

Z UNITS	MM. EXCURSION	Z UNITS	MM. EXCURSION
83	9.1	83	8.9
130	9.1	467	(9.0)
130	9.0	83	9.0
230	(9.2)*	35	8.9
83	9.0	35	8.9
57	9.0	19	8.1
57	9.0	9.5	4.2
168	(9.0)	42	8.9
168	(9.0)	83	8.9
330	(9.2)		

* Parenthesis means that at this coil distance the make shock record showed deformation.

ditions was shown. The strength of stimulus is given in Z units on the Martin scale. The magnitude of excursion is given in millimeters and the measurements are accurate to within about 0.1 mm.

It will be seen that from 39 Z to 123 Z the excursion remains constant between 5.0 mm. and 5.1 mm., the recorded variation lying within the limits of observational error. At 139 Z and 159 Z excursions of 5.2 mm. are recorded. However, at values not much above this we find evidence, to be discussed presently, that electrical disturbance other than the usual simple action current is being recorded. It seems reasonable, therefore, to conclude that the slight increase in the magnitude of excursion occurring with stimuli stronger than 123 Z does not

signify an increase in the magnitude of the action current, and that this experiment shows a limiting maximal value.

Experiment 21, in which the whole sciatic nerve was used for reflex stimulation and then for recording afferent impulses, showed a limiting maximal value even more clearly than Experiment 28, just described. In this the series of action current records was begun less than half an hour after the nerve had been exposed and ligated, and the stimulating electrodes were applied about 27 mm. from the nearest lead; the distance from the ligature was not accurately recorded, but it was considerably more than 2 cm. Table I shows measurements in chronological order from nineteen observations taken in rapid succession; beside each is shown the strength of stimulus. It is evident that a slight progressive diminution of the excursions occurred during this series. Correcting for this as nearly as possible the relation of response to stimulus will be found to follow the uppermost curve in figure 1. Constancy of response occurred here over a wider range of stimulation strengths than in Experiment 28, and, furthermore, this constancy persisted even beyond the point at which the records showed deformation. Another preparation showed no increase in the response of the peroneal nerve between 71 Z and 197 Z; still another showed practically no increase between 9.5 Z and 210 Z in the case of the peroneal nerve. Another peroneal nerve, which yielded the largest action currents obtained in any of our experiments, showed an increment of not more than 1 per cent between 13.6

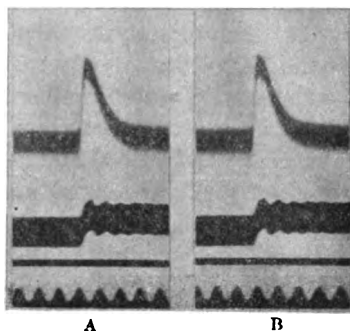


Fig. 2. Monophasic action currents, peroneal nerve. Experiment 32.

Stimuli, break shocks (coil *F*), in A, 13.6 Z; in B, 23.4 Z.

String D (see previous paper, p. 122).

In this and all other photographic records the top line shows the excursions of the string. The second line shows the time of stimulation (except when the signal magnet was disconnected). A fall in this line shows the make, a rise the break of the primary current. The small oscillations following the break are vibratory and do not indicate secondary closure of the circuit. The bottom line records time; each complete vibration = 0.01 second. In all nerve records upward excursion of string means fall of potential in proximal lead. In all experiments the nerve impulse was blocked between leads to render the action current monophasic.

Z and 23.4 Z, the records, two of which are reproduced in figure 2, being taken within about a minute of each other.¹⁷

Certain other experiments yielded curves in which the general shape closely resembles those already discussed, but in which after the approximate maximum was reached a very slight increase in the excursions persisted with further increments in the strength of stimulus. That these do not invalidate our conclusion as to a limiting maximal value is shown by an examination of Experiment 28. In this instance stimuli applied within 15 mm. of the ligature produced just such a continued gradation of excursions after an approximate maximum was reached. This is illustrated in the lowest curve in figure 1. Here an increment of response was found between 97 and 170 Z. At 558 Z no more increment appeared. Gradation here persisted up to the point at which measurements cannot be trusted because of the deformation. It has already been noted that in this experiment when the stimulating electrodes were moved 23 mm. farther from the ligature excursion of 5.0 to 5.1 mm. were obtained with all stimuli of more than 39 Z. It is quite evident that even with 558 Z applied in the region of impairment maximal stimulation of the nerve trunk was not obtained. It is presumable that a considerable number of fibers could not be excited at all in this region. The persistent gradation found in stimulating here was probably due to the great rise of threshold in many of the fibers, and perhaps also to the fact that some of the fibers couldn't be stimulated at all in this region, but only when the shocks were so strong that the spread of current reached a sufficient value at the nearest point where excitability remained.

We feel, therefore, that the conclusion is warranted that in fresh uninjured nerve a limiting maximal value to the action current is reached when the stimulus attains a certain strength. The strength of stimulus barely sufficing to give the maximum response in normal nerve was found, in the two preparations affording the best grounds for conclusions, to be in the neighborhood of 40 Z units. In the majority of our experiments impairment seems to have occurred and a very slight gradation persisted above the approximate maximum. This approximate maximum in such cases usually occurs in the neighborhood of 50 or 60 Z units. Although the value given above (40 Z) is probably the

¹⁷ In addition to these experiments done in this laboratory one of us performed a similar experiment on a cat's peroneal nerve with Dr. H. B. Williams, in the Physiological Laboratory of the College of Physicians and Surgeons in New York. This experiment also gave a result similar to those recorded here.

normal maximal value for the uninjured nerve trunk, it must be remembered that in most physiological researches nerves are subjected to a good deal of pressure in dissection, especially when blunt instruments are used, and are often ligated near the point of stimulation. In such work, therefore, electrical recording would probably show a condition more like the majority of our experiments, with a higher approximate maximal value of stimulus and a somewhat persistent grading of response.

B. Deformation of the action current record

In our experiments we have found the galvanometer records to show only the characteristic curve of the action current with both make and break induction shocks as long as these shocks were kept below a certain range of values (150-300 Z units in the case of break shocks); but with stronger shocks the curves have shown deformation which becomes increasingly marked as long as the strength of the shocks is increased. Figures 3 and 4 show several series of such irregularities, together with normal submaximal and maximal responses for comparison. Make as well as break shocks are shown, and it will be noted that the former cause deformation usually at more remote coil-distances than the latter, although the make shocks under the conditions obtaining in the primary circuit are about one-eighth as powerful physiologically as those of break shocks at the same coil-distances, if we may rely on evaluation by thresholds.¹⁸ In view of the approximate uniformity of experimental conditions,¹⁹ the variety presented by the records is rather striking. In some it will be seen that the deformation consists chiefly in a sharp preliminary notch (e.g., figure 3, row B, No. 4, row C, No. 5 break shocks) similar to that shown in some of Gotch's capillary electrometer records. In some few of the records (e.g., figure 3, row B, No. 3; figure 4, lower row, No. 3) the only anomaly consists in a second excursion of the string, suggesting as its cause a second impulse in the nerve. Garten has made a similar observation which he interprets in this way.²⁰ Theoretical reasons for assigning some degree of probability to this interpretation will be considered further on. Several of the records are too complex to be analyzed without far more data than we have been able to secure. We have obtained some evidence, however, which tends towards their elucidation.

¹⁸ See Martin: *Op. cit.*, 102.

¹⁹ Exception to this is noted below and in the legends to figures 3 and 4.

²⁰ Garten: *Zeitschr. f. Biol.*, 1909, lii, 552, and figure 17.

a. *The electrical artefact.* In the literature of electro-physiology it is not uncommon to find mention of the "escape of current" into the recording apparatus. For instance, Gotch and Burch,²¹ referring to their records of the action currents of nerves taken with the capillary electrometer, mention sharp notches in the curves as "due to an escape

Fig. 3. Simple and deformed action current records compared. Each horizontal row shows a series of observations from a single experiment without shift of electrode contact; each space between records denotes change of coil distance; stimuli are progressively stronger from left to right in each row. Two speeds of film were used. To aid comparison some records at both speeds, otherwise identical, are put side by side. In all except row D (as in all other nerve records in this paper) the proximal lead was connected with the upper end of the string. In this case the leads were reversed; also the magnet current.

In the following, each group of records at one coil distance is designated with a single number; coil distances are given in millimeters, Z units refer to break shocks (make shocks not evaluated). Coil *F* used in all. Current in primary coil given in amperes (the same throughout each experiment). The response was rendered monophasic by crush in all but Experiment 5.

Row A, Experiment 5, peroneal nerve. String C. High-voltage magnet coil. Nerve devitalized by heat under distal lead.

Shunt in primary circuit (see previous paper, p. 138), 0.105 amp. Martin knife-blade key. 1, 250 mm., $\frac{11}{K}$ Z; 2, 150 mm., $\frac{99}{K}$ Z; 3, 90 mm., $\frac{465}{K}$ Z; 4, 80 mm., $\frac{506}{K}$ Z; 5, 70 mm., $\frac{538}{K}$ Z; 6, 40 mm., $\frac{598}{K}$ Z; 7, 0 mm., $\frac{662}{K}$ Z.

Row B, Experiment 8, peroneal nerve. String D. High-voltage magnet coil. No shunt in primary circuit; 0.226 amp. Knife-blade key. 1, 370 mm., 6.8 Z; 2, 160 mm., 164 Z; 3, 120 mm., 650 Z; 4, 0 mm., 1580 Z.

Row C, Experiment 9, peroneal nerve. String D. High-voltage coil. 0.284 amp. Knife-blade key. 1, 360 mm., 9.5 Z; 2, 160 mm., 210 Z; 3, 140 mm., 425 Z; 4, 100 mm., 1260 Z; 5, 0 mm., 2020 Z.

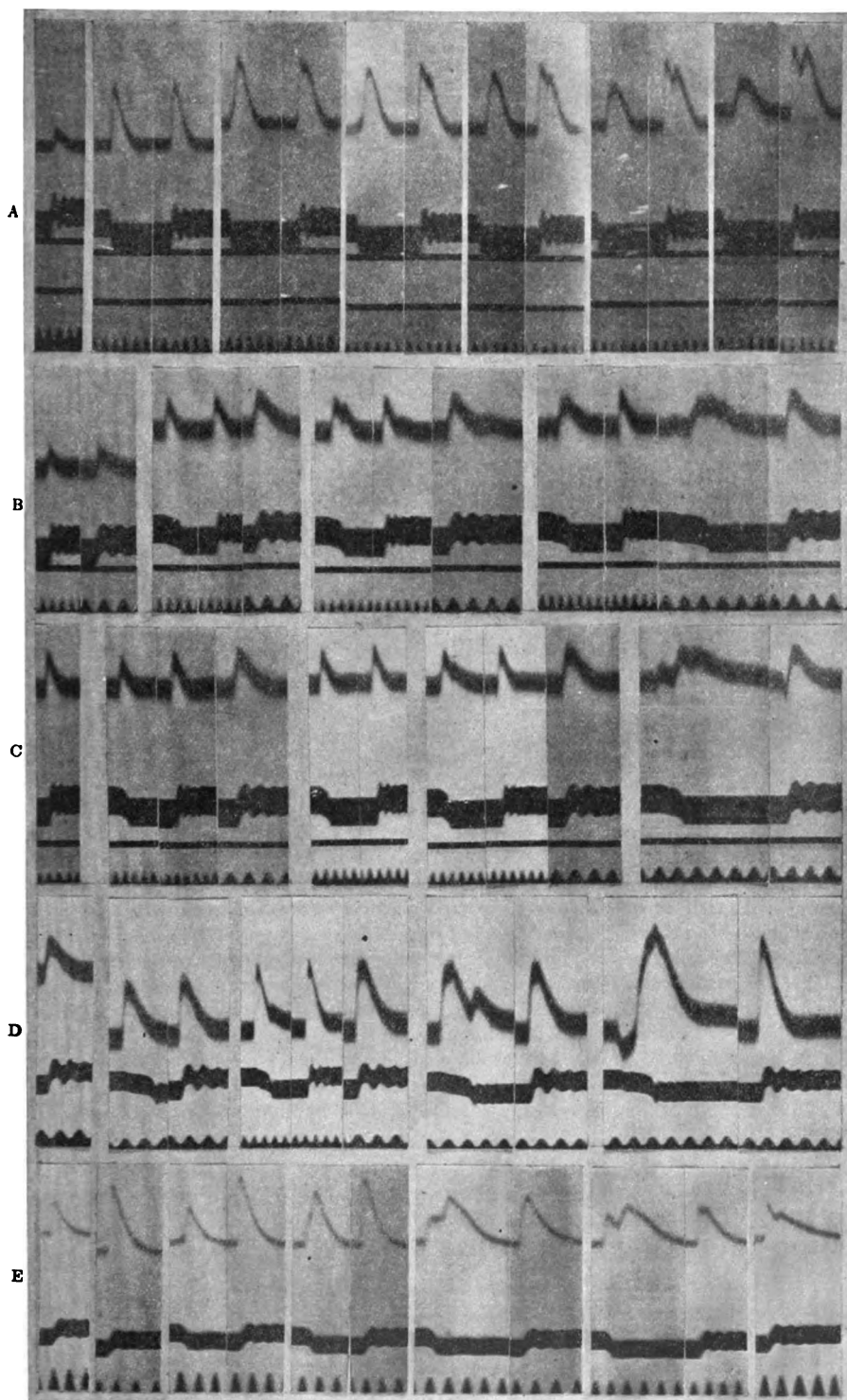
Row D, Experiment 17, popliteal nerve in moist chamber. String D. High-voltage coil. 0.222 amp. Copper point and mercury key (see previous paper, p. 132). 1, 360 mm., 7.4 Z; 2, 180 mm., 93 Z; 3, 150 mm., 236 Z; 4, 140 mm., 334 Z; 5, 0 mm., 1570 Z.

Row E, Experiment 21, sciatic nerve. String E. Low-voltage magnet coil. 0.306 amp. Copper point and mercury key. 1, 370 mm., 9.5 Z; 2, 180 mm., 130 Z; 3, 170 mm., 168 Z; 4, 140 mm., 467 Z; 5, 80 mm., 1690 Z; 6, 50 mm., 1950 Z; 7, 0 mm., 2200 Z.

The first four break shocks in Row E furnished measurements recorded in Table I.

For calibration curves, see figure 5. The knife-blade key when used in these experiments gave clean makes and breaks.

²¹ Gotch and Burch: Loc. cit., 413.



from . . . the induction shocks used for excitation." Lucas has used such notches as evidence of the exact time of stimulation.²² The exact nature of this "escape of current" is not stated in those papers in which we have found it mentioned, but it is our impression that many physiologists understand the term to imply a condition in

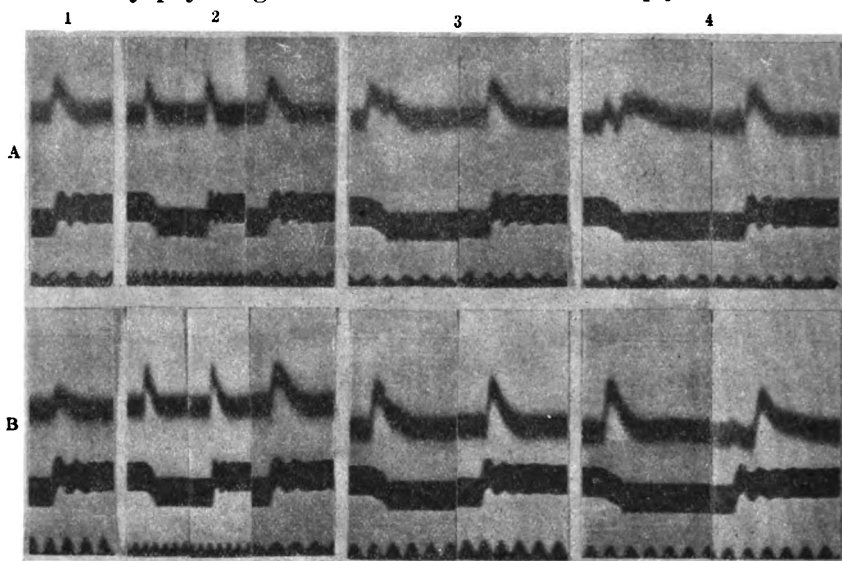


Fig. 4. Same plan of arrangement as in figure 3. Coil *F*. String *D*. High-voltage magnet coil. In *A* the kathode of the secondary coil on break shock was connected with the stimulating electrode nearest the galvanometer leads, as was the case in all experiments when not otherwise stated. In *B* the stimulating electrodes were reversed. The difference between the two experiments supplements the evidence in figure 15.

A, Experiment 30, popliteal nerve. 0.280 amp. Knife-blade key. 1, 310 mm., 15.6 Z; 2, 180 mm., 119 Z; 3, 140 mm., 425 Z; 4, 0 mm., 2000 Z.

B, Experiment 31, sciatic nerve removed under ether, kept 3 hours in Ringer solution, room temperature; then in moist chamber 4 hours before observations. 0.278 amp. Knife-blade key. 1, 250 mm., 32 Z; 2, 160 mm., 207 Z; 3, 120 mm., 816 Z; 4, 0 mm., 1990 Z.

which the leading-off electrodes are so placed in relation to the tissue that the recording circuit can provide a path for a portion of the current which passes between the two stimulating electrodes as in figure 6. That this does not represent the distribution of electrical disturb-

²² Lucas: *Journ. Physiol.*, 1911, xliii, 51 and figure 2, 52.

ance in such arrangements as we have dealt with, we have both theoretical and experimental evidence.

It is not necessary that the recording circuit shall provide a path for the current flowing between the stimulating electrodes, nor that one of the leads should be very close to one of these in order that appreciable electrical disturbances resulting directly from the stimulating cur-

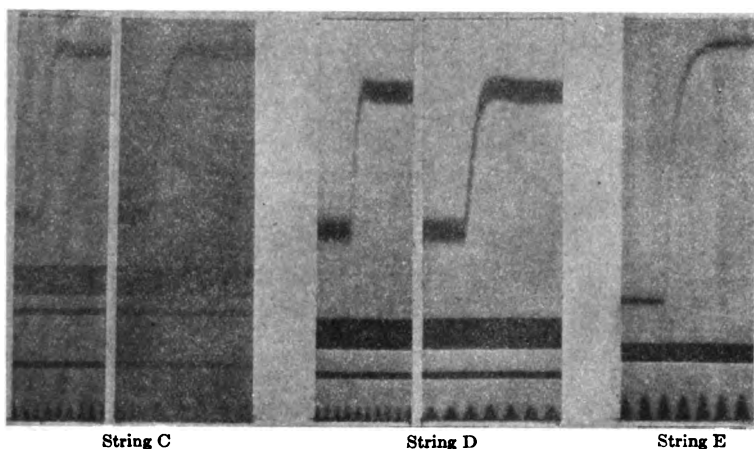


Fig. 5. Calibration curves. These show typical excursions of the three strings employed, on the make of a constant current. The tension in the case of Strings C and D was the same as was regularly employed in all experiments with the string in question (see previous paper, p. 122); in the case of String E it was the same as in the records in figure 3, E. The resistances were of the same order of magnitude as was commonly found in our experiments; with String D it was identical with that found in the nerve circuit used for figure 3, B. The E.M.F. and resistance in series with the string in each were as follows:

$$\begin{array}{ll} \text{String C: } \frac{10 \text{ millivolts}}{\text{string} + 46,000 \text{ ohms}} & \text{String D: } \frac{10 \text{ millivolts}}{\text{string} + 38,000 \text{ ohms}} \\ \text{String E: } \frac{10 \text{ millivolts}}{\text{string} + 25,000 \text{ ohms}} & \end{array}$$

rent may appear in the recording instrument. It is an elementary fact in electricity that if the potential of any point on a conductor is suddenly altered a transient current flows between that point and the rest of the conductor, tending to equalize the potential throughout. This current at any point varies in magnitude according to the electrostatic capacity to which the conducting path under consideration leads. Every conductor has some capacity and any portion having large ca-

capacity will provide a sort of reservoir to which, under the conditions assumed, an appreciable current will flow. Now it has been pointed out by Williams and Crehore²³ that a nerve trunk, on account of its structure, must have considerable electrostatic capacity. Furthermore, most pieces of recording apparatus have appreciable capacity; this is notably so in the string galvanometer of the Cambridge make (used by us), in which one end of the string is connected with the core of the magnet. When, therefore, the potential of a nerve is suddenly lowered²⁴ at the point of stimulation a transient current flows to this point from all remote portions of the nerve and from such capacity as may be connected thereto in the form of recording apparatus. The general distribution of lines of current flow may be indicated schematically in figure 7. In determining the nature of the transient current through the string the distribution of capacity in various parts of the connected system of conductors is therefore of considerable importance.

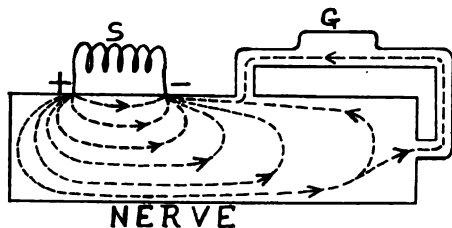


Fig. 6. *S*, stimulating inductorium; *G*, recording instrument (galvanometer or electrometer).

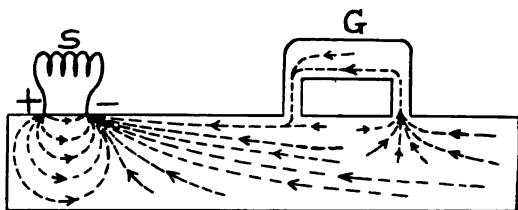


Fig. 7. *S*, stimulating inductorium; *G*, galvanometer. Capacity is roughly represented by enclosed area.

Referring to figure 7 in which is shown a rough schematic representation of the capacities involved in our experiments, it will be noted that there is capacity distributed throughout the length of the nerve trunk as well as in the galvanometer. The capacity of the galvanometer is chiefly in the iron core and this is metallically connected with the upper end of the string. In all of our experiments with nerves under direct stimulation, except that recorded in the fourth row (D) of figure 3, the proximal lead (i.e., the electrode on the nerve nearest to the stimulating electrodes) was connected with the upper end of the string, the distal lead with the lower; in this one case the usual connections were reversed as was also the current exciting the galva-

²³ Crehore and Williams: *Proc. Soc. for Exper. Biol. and Med.*, 1913, xi, 59.

²⁴ We choose as our example the lowering instead of the raising of potential, since this is what occurs at the stimulating electrode.

nometer magnet.²⁵ Thus in all but one of the experiments shown in figures 3 and 4, the galvanometer capacity was proximal to the string. much depends on the position, in relation to the electrodes, of the maximum capacity of the nerve trunk. Thus, if the capacity of that portion lying beyond the distal lead be far greater than that of the galvanometer, the transient current will flow through the string in the direction indicated in figure 7 (assuming the potential of the stimulating electrode nearest the lead to have been lowered). If the greater part of the total capacity be in the magnet core, the transient current will flow in the opposite direction through the string. Briefly, the direction of the transient currents in the string depends on whether the capacity of that portion of the nerve which is more accessible to

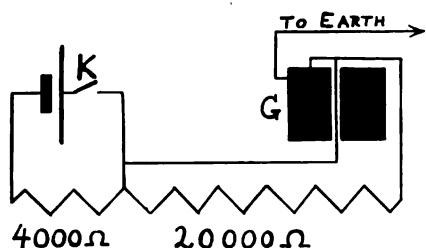


Fig. 8. K, key; G, galvanometer.

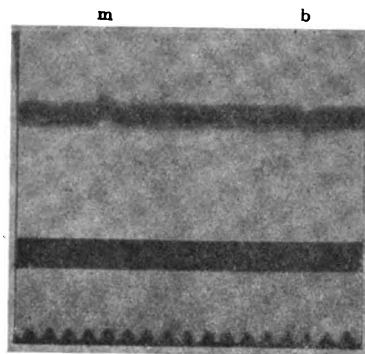


Fig. 9. Transient effects from make (m) and break (b) of constant current. String D. Wiring shown in figure 8. Platinum contact key.

the spreading current by way of the galvanometer circuit than by way of the nerve itself bears more or less than some fixed ratio to the capacity of the magnet core. Thus, we might expect to find the transient effect occurring in opposite directions in different experiments unless the nerves are always identical in size and capacity and identically arranged.

In order to show the presence and nature of the transient currents in circuits uncomplicated by action currents, two methods were employed;

²⁵ This was reversed in order to make the action current deflect the string in the usual direction.

one was the substitution of blank resistances for the nerve, making these of the same order of magnitude as is commonly found in nerve, and the other was the use of a dead or inactive nerve in place of an active one. The experiments with the blank resistance will be described first.

The simplest condition studied was that of a galvanic current derived from a single galvanic cell. A 4000 ohm spool was connected with a dry cell and a key; a 20,000 ohm non-inductive resistance was connected with the galvanometer terminals; the string terminal not connected with the magnet core was also connected with one end of the 4000 ohm spool; the magnet core was put to earth. The arrangement is shown in figure 8. Making and breaking the circuit through the

4000 ohm spool caused transient excursions of the string of which examples are recorded in figure 9.

Conditions more closely resembling those obtaining in our experiments were studied by replacing the dry cell with an inductorium. In this experiment a small 5000 ohm spool was introduced between the terminals of the secondary coil. The 20,000 ohm segment of a non-inductive resistance box was connected with the terminals of the galvanometer, and the adjacent 30,000 ohm segment of

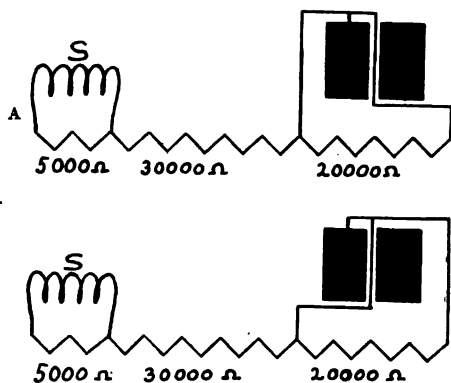


Fig. 10. S, secondary coil of inductorium.

the same box was used to connect one or other terminal of the galvanometer with that terminal of the secondary coil known to be cathode on the break shock. Figure 10 shows the arrangement of apparatus in this experiment. A, with the upper end of the string proximal, represents the usual condition in our experiments with nerves. B, with the wires reversed, represents the arrangement in Experiment 17 (shown in fig. 3, D) with the exception that in the experiment with the blank resistance the current exciting the magnet was not reversed as it was in Experiment 17. Figure 11 shows the excursions of the string resulting from make and break shocks, with the secondary coil at zero (i.e., completely covering the primary), in both arrangements of the circuit, and in each case

with and without the magnet core being put to earth.²⁶ The excursions differ from those obtained with a galvanic cell in that they show, as is to be expected, that the transient current is diphasic, i.e., flows first in one direction, then in the other through the string. When the core is not to earth the excursions differ in the two arrangements chiefly in that they are in opposite directions, but they also differ slightly in size, that with the upper end of the string distal being the larger. When the magnet core is put to earth this difference in magnitude is greatly increased, the excursion being almost abolished when the earthed end of the string is proximal and much augmented when it is distal. The initial deflection in each case indicates that the potential of the end of the string connected with the proximal lead has changed in the same

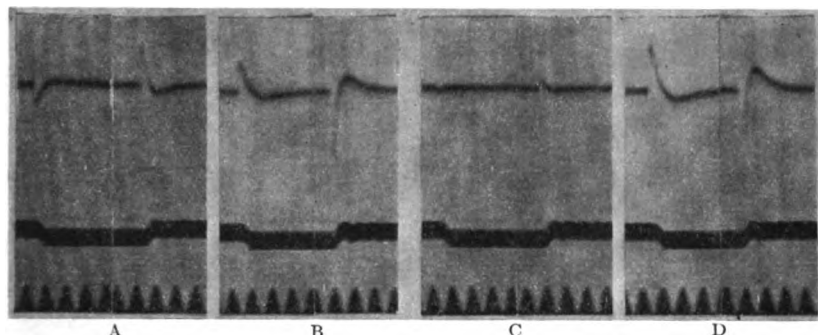


Fig. 11. Diphasic transient effects from induction shocks. In A and C the wiring was as shown in figure 10, A; in B and D it was as in figure 10, B. In A and B the magnet core was not put to earth; in C and D it was. String E. Tension, 1 cm. excursion = 12.4×10^{-8} ampere; i.e., twice that used in the calibration curve (fig. 5). Copper point and mercury key.

sense as has the terminal of the secondary coil nearest to the galvanometer; i.e., the end of the string nearest the induction coil is negative when the negative terminal of the inductorium is nearest the string. The difference in magnitude between the excursions in A and B (fig. 11) depends upon the capacity of the magnet core. An important difference between the electrical arrangements in this artificial system

²⁶ In this and all subsequent experiments the galvanometer was equipped with the low-voltage magnet coil excited by 8 Edison storage cells. In the experiment shown in figures 8 and 9 the high-voltage magnet coil was used and was excited by the current from the local power plant. Cf. Forbes and Gregg: *Loc. cit.*, 121.

and in those involving a nerve trunk is that with the nerve non-polarizable "boot" electrodes are interposed between the nerve and the galvanometer, and these have resistances of several hundred ohms, often over a thousand. The significance of this is that if the principal capacity were in the magnet core no appreciable current would flow to it by way of the string in a system such as is shown in figure 10, A, for on the principle of divided circuits practically all the current would flow through the proximal lead wire which has a negligible resistance compared with the other path. On the other hand, with an electrode resistance such as is interposed in a physiological circuit, an appreciable fraction of the total transient current would flow through the string on its way to the core.²⁷

The other method used for examining these electrical disturbances, which we may for convenience call "artefacts," was the introduction of nerves which were dead or at least physiologically inactive. Three such were studied. One was a peroneal nerve which after removal from the animal's body was kept for two days in Ringer solution at room temperature, after which it showed no trace of action current on stimulation. Another nerve was removed from an animal immediately after decerebration under excessively profound ether anaesthesia, and for some reason failed to exhibit any action currents during the next hour when it was examined. That the nerve was not dead was shown by the fact that after it had been washed for over an hour in Ringer solution it exhibited action currents which were nearly normal. The third nerve (popliteal) was killed by immersing it in Ringer solution heated to 70°C.

The first of these nerves had yielded normal action currents when fresh, and after 19 hours in Ringer solution, yielded action currents still more than half as large as when fresh. After 44 hours in Ringer solution it was arranged with 4 cm. intervening between the stimulating electrodes and the nearest lead to the galvanometer. No excursion of the string occurred until the secondary coil was brought close enough to give a break shock of 620 Z units. This produced an excursion barely discernible in the record. Make and break shocks with the secondary at zero (break shock, 2000 Z units) produced excur-

²⁷ One experiment was performed with graphite lines on ground glass for resistance, and a galvanic cell instead of an induction coil, and the resistances were arranged to be closely comparable with those of nerve and electrodes as ordinarily set up. No excursions at all resulted from opening or closing the galvanic circuit; probably because the capacity of the conductor was too small.

sions shown in figure 12, *A*. The stimulating electrodes were then moved to within 12 mm. of the proximal lead and shocks of the same strength produced the greatly augmented excursions shown in figure 12, *B*.

The nerve whose functional activity was temporarily suspended was arranged with the galvanometer leads 35 mm. apart (the usual distance was about 25 mm.), and the stimulating electrodes only 15 mm. from the proximal lead. With this arrangement the electrical "artefact" appeared with weaker shocks than usual. Figure 13 shows the disturbances evoked with coil distances at which break shocks amounted to 147 Z (in *A*) and 372 Z (in *B*). When the stimulating electrodes were placed 55 mm. away from the proximal lead no excursions of the string resulted from these shocks; stronger shocks were not tried.

The nerve which was killed by immersion in hot Ringer solution yielded with powerful shocks very small excursions similar to those in figure 13, *A*, but opposite in direction.

It is interesting to note that in these experiments with non-functional nerves the current through the string is in the same direction as in the case

of the blank resistance, except in the case of the nerve killed in hot Ringer. It should also be noted that a small notch in the record from a make shock taken from this nerve just before it was heated, shows a transient current in the same direction as in the make shocks with the blank resistance and with the other inactive nerves. There appears to have been a reversal of direction in the transient current correlated with heating the nerve. A possible explanation might lie in the reduction of capacity which is a very probable consequence of destroying the nerve structure with heat. If the nerve's capacity were so reduced that the greater part of the capacity of the entire system was in the magnet core, we should expect such a reversal of the tran-

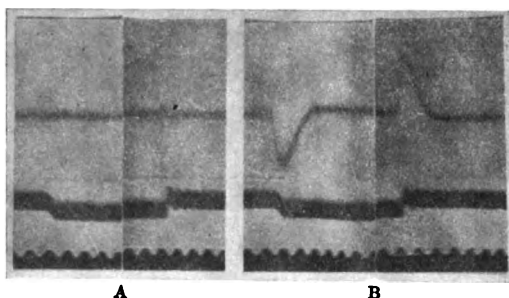


Fig. 12. Transient effects in dead nerve (see text). String E. Tension the same as in figure 11. Low-voltage magnet coil; core not grounded. Galvanometer leads 25 mm. apart on the nerve. Experiment 19. Cu and Hg key.

sient current through the string. The length of nerve beyond the distal lead was not recorded in these experiments as its possible significance had not become apparent to us at the time the experiments were performed.

In all these cases of transient current arising from induction shocks in which we used inert conductors, both in the case of the blank resistance and in that of the inactive nerve, the excursions are of a simple character. It is difficult to see how some of the more complex curves we have recorded from active nerves stimulated with powerful shocks can be interpreted as compounds of the simple electrical "artefact" and the equally simple monophasic action current. In attempting to determine as far as possible the nature of the disturbances, we made use of the latencies under various conditions of stimulation. Meas-

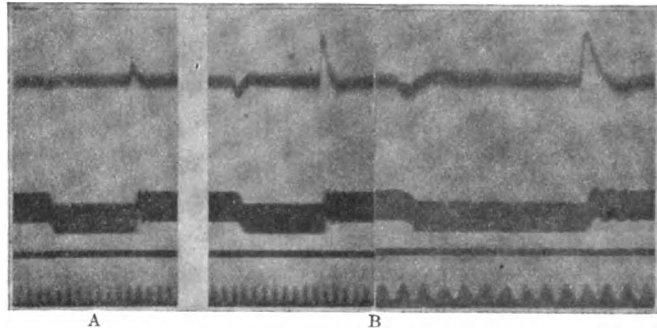


Fig. 13. Inactive nerve (peroneal), see text. String C. High-voltage magnet coil. Both speeds of film shown in B, procedure otherwise identical in these two pairs of shocks. Experiment 7. Knife-blade key.

urements were made on the films of the time interval between the first motion of the signal magnet and the first deflection of the string. In such measurements our accuracy was limited by the degree of accuracy with which time could be measured (readings being possible only to within about 0.4σ of the true value) and by the slight lag of the signal magnet which, as stated in our previous paper, appeared to vary slightly and to have a mean value of about 0.6σ . Considering the shortness of latency in recording the response of a nerve to direct stimulation, the percentage error is necessarily large. Consistent and significant results were, however, obtained from these measurements. Submaximal stimuli (ranging from 7 to 40 Z) give latencies varying according to the distance from the stimulating electrodes to the proxi-

mal lead and indicating a velocity of the nerve impulse in the neighborhood of 20 or 30 meters per second. For instance, in Experiment 28, with a distance of 50 mm. a submaximal response followed a latency of about 2σ . In general, when the stimuli were increased greatly in strength the latency became clearly less; in some preparations a diminution was evident with shocks of less than 100 Z, in others it was not apparent until a value of about 200 Z was reached. With shocks amounting to several hundred Z units the latency was reduced to within the limits of observational error. Whenever a preliminary notch was present which was clearly distinguishable from the action current, it was so nearly simultaneous with the first motion of the signal magnet that no interval could be detected with certainty.

A transient current passing through the string in the manner just analyzed and attaining sufficient magnitude to produce an appreciable excursion of the string, would naturally deform the curve in the resulting record, and since it necessarily occurs practically at the instant of the break of the current, such deformation will precede the part of the curve due to the action current proper. In those cases in which no such clearly defined notch occurred and in which, nevertheless, the latency was clearly diminished, the question arises whether the first excursion is due to any such electrical "artefact" or to a true action current appearing after a briefer interval from the stimulus than usual. Many records of action currents with shortened latency but showing no deformation whatever and produced with stimuli between 100 and 200 Z units lead us to suppose that the latter is the case, that we are dealing with an unusually prompt action current and not an "artefact." Such unusual brevity of the latency does not necessarily imply an unusually high velocity of the nerve impulse, for it can be explained as a result of the spread of electrical disturbance along the nerve trunk. Referring again to figure 7 it will be seen that there is a general convergence of lines of current flow toward the stimulating electrode not only from the portions of the nerve lying in the direction of the other terminal of the inductorium but also from all other portions of the nerve in proportion to their capacity. As these lines of transient current flow traverse the individual fibers there will be an infinite series of physiological anodes and kathodes. If the shock be strong enough, physiological kathodes, at which the current flow becomes sufficiently intense to stimulate the fibers, will be found at a considerable distance along the nerve from the stimulating electrode. If this is the case, excitation will occur at these points at the instant of stimulation and

the impulse will have less distance to travel before reaching the proximal lead. In this way the observed shortening of latency may readily be explained.

b. The effect of the signal magnet. Certain observations in other experiments with the apparatus revealed the possibility of transient currents resulting in part from the arrangement of our signal magnet. As was stated in our previous paper the wires to the signal magnet in the primary circuit of the inductorium were enclosed in lead sheathing and the lead put to earth. This introduced an appreciable capacity in the primary circuit. It is as if the primary circuit were connected

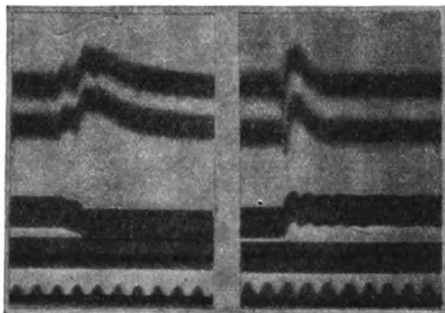


Fig. 14. Showing removal of signal magnet (see text). The lower galvanometer curve in each is the one made without the signal magnet. With signal, primary current 0.280 amp., break shock 2020 Z; without signal, 0.290 amp., 2080 Z. Experiment 9. (The curves made with the signal are duplicates of those in figure 3, C 5.) Knife-blade key.

with one plate of a condenser of which the other plate is put to earth. To determine what part this might play in the deformation of the records powerful shocks, with the secondary coil at zero, were used to stimulate a nerve, the signal magnet being included in the primary circuit as usual, and the responses to both make and break shocks were recorded. Then the signal magnet was disconnected from the primary circuit and in its place was introduced an approximately equal resistance in order to keep the primary current the same. Again make and break shocks, being of substantially

the same intensity, were recorded. The curves traced by the string were almost identical in the two conditions. To render this evident we have reproduced the records together (fig. 14) by superposing the films and printing through both. Evidently the signal magnet with the capacity involved contributes practically nothing to the deformation of the action current records caused by powerful shocks.

c. Reversal of the direction of shock. In two experiments the effect of reversing the wires from the secondary coil to the stimulating electrodes was examined. The results of these experiments are shown in figure 15. In each case the secondary coil was at zero. In the upper

row (showing an experiment with coil *F*) the value of the break shocks was about 2000 Z units. Figure 15, *A*, shows both make and break shocks with the usual wiring, the kathode on the break shock being nearest the galvanometer. *B* shows similar shocks in the reverse directions. The lower row shows an experiment with a short coil (not

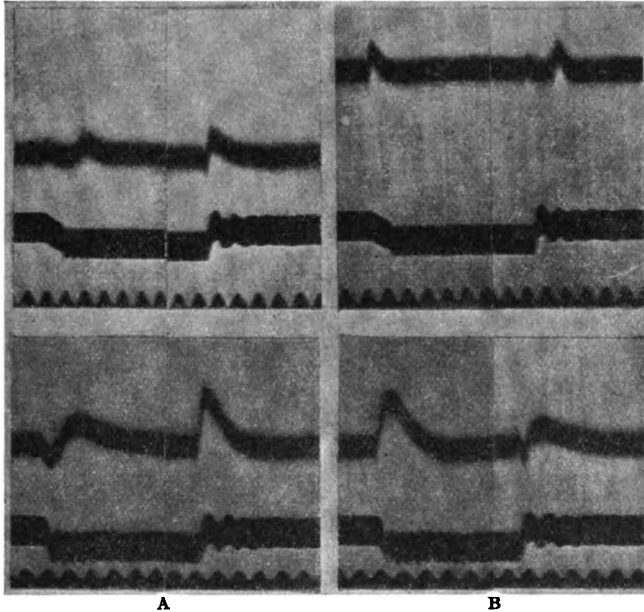


Fig. 15. Description in text. String D. High-voltage magnet coil. Upper row, Experiment 31. Sciatic nerve, the same one as in figure 4, B, after being kept over night (14 hours) in Ringer solution at room temperature. Break shocks each 1900 Z. Knife-blade key. The overnight impairment may be seen by comparing the make and break in B with those in figure 4, B 4, in which the shocks are the same; the nerve was replaced in as nearly as possible the same position on the electrodes in the morning as on the night before. Lower row, Experiment 14. Cu and Hg key. The insulation at one point in the magnet coil having burned out just before this experiment, it was only possible to use the 110-volt current, instead of the usual 220-volt current, to excite the galvanometer magnet (cf. previous paper, p. 138, legend to fig. 6).

calibrated) whose polarity had not been determined. It will be seen that the shapes of the curves are similar when the shocks are in the same direction regardless of whether they are make shocks or break shocks; yet appreciable differences exist between "ascending" make

and "ascending" break and between "descending" make and "descending" break.²⁸

d. The influence of the iron core. A few attempts were made to see what effect the presence of an iron core in the inductorium might have on the nature of the deformation of action current records. To this end, since coil *F* was so constructed that the core could not be removed, another having a movable core, was employed. This coil, which we

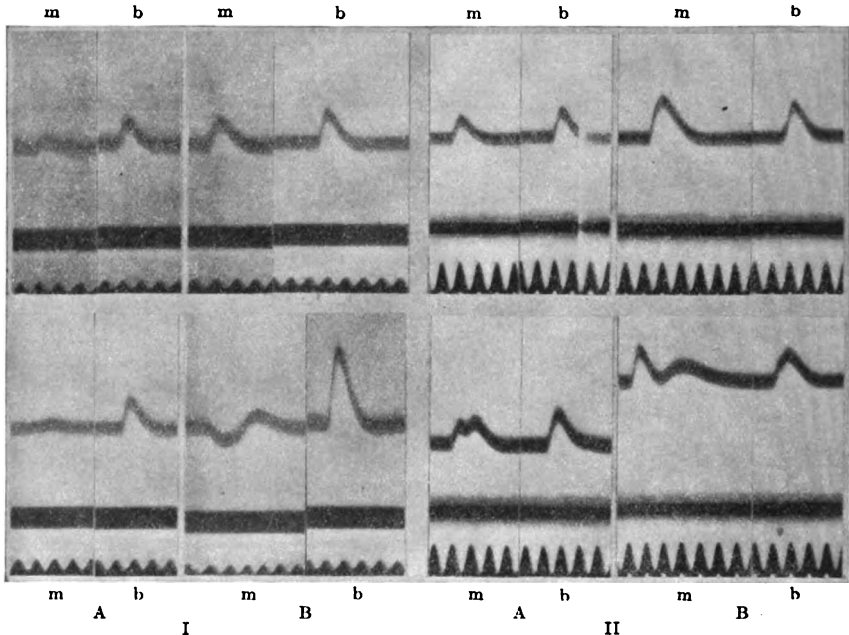


Fig. 16. Influence of iron core. Coil *X*, polarity not determined. Cu and Hg key. String C. Low-voltage magnet coil. Core not grounded. Primary current in both experiments about 1.5 amp. Lower row, iron core in coil; upper row, core removed. Sciatic nerve used in each; and placed in moist chamber. I, Experiment 27. II, Experiment 28. Differences described in text.

In both I and II, A was made with coil distance, 95 mm., B, coil distance, 0 mm. Approximate strengths of break shocks as follows:

Upper row: A, 104 Z; B, 2200 Z. Lower row: A, 915 Z; B, 7800 Z.

designate coil *X*, is short, the primary and secondary each being only about 7 cm. long, and is provided with bundles of soft iron rods tied together to make a compact and readily movable core. Comparative series of stimuli were studied with this coil both with and without the

²⁸ Cf. figure 4, lower row and explanation in legend.

iron core and the results were compared with those of stimuli derived from coil *F* and applied to the same preparation. A rough calibration of coil *X* was made, both with and without the core, by comparing the primary currents required to give threshold stimulation to a frog's muscle at different coil distances, and standardizing the values with a coil previously calibrated by Dr. Martin. Without taking the time for a very careful calibration of this coil we were still able to obtain a fair idea of the approximate value of our stimuli as judged by the criterion of thresholds.

Figure 16 shows the results of two experiments with different preparations but with substantially the same procedure. The only differences noted were that in Experiment 28 (second half of fig. 16) the nerve was in better condition than in Experiment 27, and the whole preparation (electrodes and nerve) had a lower resistance, 18,000 ohms as compared with 50,000. It is probable that both the nerve and the porous "boot" electrodes were drier in Experiment 27. In Experiment 27 the stimulus was applied 36 mm., from the proximal lead, while in this part of Experiment 28 it was applied 50 mm., from the proximal lead. In all other respects as far as details were noted the procedure was the same. In the upper row are shown the records obtained with coil *X* without the iron core. Immediately below each of these is the record obtained under identical conditions and with the same position of the secondary coil, but with the iron core. It should be noted that only one record in the case of Experiment 27 shows deformation, while with shocks of precisely the same strength in Experiment 28 all but one show notable deformation. This difference may be explained by the greater dryness of the nerve and the higher resistance in the circuit. It is also notable that the deformations in the two experiments are quite different in character.

It appears from a comparison of these records that the deformation, especially on make shocks, is much more marked when the core is present than when it is not. But it must be remembered that the shocks are (as indicated in the legends of the figures) far more powerful, other things equal, when the core is present, and that therefore the increase in deformation might be the result of increased intensity and not due to any peculiar effect of the core as such. To make a valid comparison it is desirable to use shocks of as nearly as possible the same intensity, offsetting the intensification due to the core by either weakening the primary current or by increasing the coil distance. No strict comparison of this sort was made with coil *X* but it may be

noted that in Experiment 28 (fig. 16) the deformation on the make shock is far more marked with the core in and a coil distance of 95 mm., than with the core out and the secondary coil at zero, while the difference between break shocks under corresponding conditions is comparatively slight. We have not determined the necessary constant "C" for evaluating make shocks, but the approximate values of the break shocks in the two cases are 915 Z and 2180 Z respectively, much greater in the arrangement with which the made shock produces the smaller deformation. Thus records tend to show that the iron core is an important factor in the causation of the more elaborate deformations. Figure 17 shows a pair of shocks, make and break, from coil *F* in Experiment 28, recorded immediately before those from coil *X* shown in figure 16 *II*. The break shock here amounts to 2080 Z which is, within

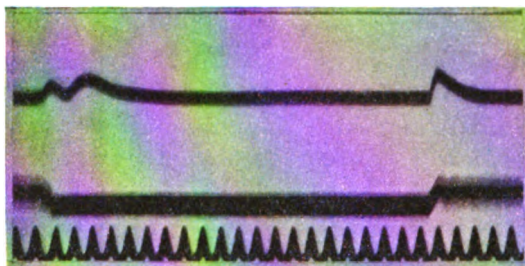


Fig. 17. Experiment 28. Coil *F*. (See text.) Primary current, 0.285 amp. Break shock, 2080 Z. Usual wiring and direction of shock. Cu and Hg key. Compare with figure 16, *II*.

the limits of accuracy in our calibration, equal to the break shock produced by coil *X* with the core out and the secondary at zero (fig. 16, *II B*, upper row). Here again the make shocks differ greatly, but we have not the data for estimating their relative values by the criterion of thresholds.

Figure 18 shows two series of records also bearing on the influence of the iron core. They were obtained with corresponding shocks in the two parts of Experiment 28, the stimulating electrodes being 50 mm. and 27 mm. respectively from the proximal leads. Here, as in other experiments, it is evident that the deformation becomes more marked when the stimulating electrodes are near than when remote from the leads.

It is noteworthy that the highly complex curves resulting from powerful shocks are only obtained when an iron core is present in the coil. The most powerful shocks obtained without the core, even when applied close to the leads, produce only a bending of the first limb of the curve (away from the base line on the make, toward it on the break) such as we might expect from a simple transient current practically synchronous with the action current. It seems, then, that the peculiar

phenomena denoted by the complex curves shown are in some way associated with the iron core of the inductorium.

e. Double or compound stimulation. We have already noted that the deformation in some of our records suggests as its cause a second impulse in the nerve, and that Garten construed a similar observation in this way.²⁹ In order to consider properly the theoretical possibility of such an occurrence we must refer to Nernst's theory of electrical

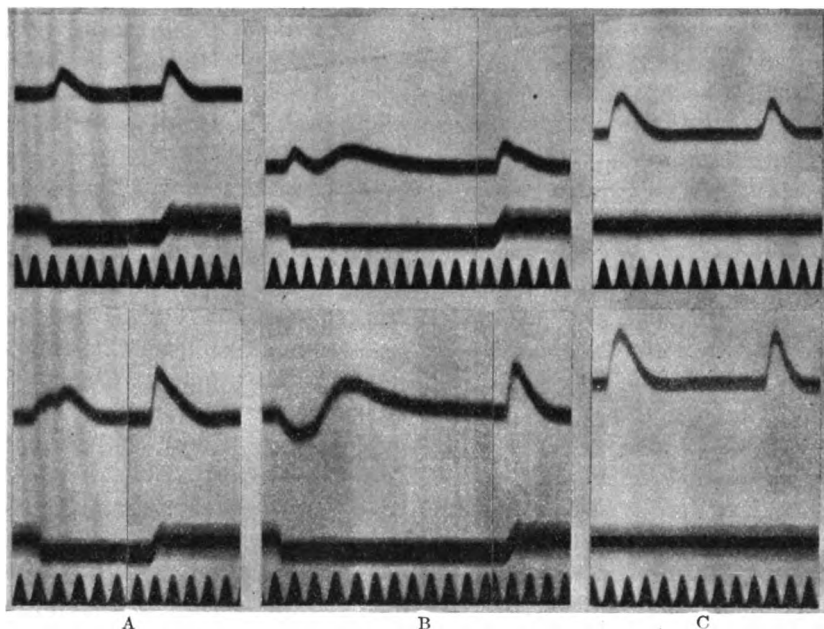


Fig. 18. Experiment 28, upper row, stimulus 50 mm. from proximal lead; lower row, stimulus 27 mm., from proximal lead.

In both rows: A and B, Coil *F*. Primary current, 0.49 amp. C, Coil *X*. Primary current about 1.5 amp.; no iron core.

A, coil distance, 123 mm., break shock, 1400 Z; B, coil distance, 0 mm., break shock, 3700 Z; C, coil distance, 0 mm., break shock, 2200 Z.

In C, the records are continuous between make and break shocks. In all Cu and Hg key.

excitation which has been modified by Hill,³⁰ and which in its modified form has been shown by Lucas³¹ to afford a most satisfactory basis for

²⁹ Garten: Loc. cit.

³⁰ Hill: Journ. Physiol., 1910, xl, 190.

³¹ Lucas: Journ. Physiol., 1910, xl, 225.

interpreting the observed relations between the strength and duration of current required to excite various tissues. As summarized by Lucas,³² this theory is based on the assumption that an exciting current produces its effect by concentrating certain of the ions by which it is carried at membranes (impermeable to the ions) contained within the excitable cells. In his modification of the theory Hill, in order to explain the relative inefficacy as stimuli of slowly increasing currents, has further assumed that the propagated disturbance is initiated by the breakdown of some unstable substance in the cell, normally in equilibrium, but acted on by the ions involved in excitation when these are more concentrated than normal. He assumes that when the ions are sufficiently concentrated (the requisite degree of concentration varying with the amount of unstable substance present) the reaction proceeds explosively, much as hydrogen and oxygen combine when heated together above a certain temperature.

The theory avowedly deals only with the conditions necessary at the point of stimulation for starting the propagated disturbance, and does not attempt to explain the nature of the latter. It gives us no clue to the refractory phase or the related fact that excitable tissues respond rhythmically and not continuously to continuous stimuli. It has been shown by Garten³³ that a constant current applied to a motor nerve can produce a rhythmic response in the nerve or in the innervated muscle, in fact, the "kathodal closing tetanus" is a classical observation.³⁴ It is also a familiar fact that the amphibian heart, brought to a standstill by a Stannius ligature, will respond with rhythmic contractions to continuous stimulation either with a rapid series of induction shocks or with a constant current. Just how recovery from a preceding response occurs and why the continuous stimulus produces rhythmic response are matters which appear not to have been explained. Following Hill's hypothesis we may suppose that recovery depends on the renewal of the unstable substance. But how this substance is protected by a refractory period from the action of the ions and permitted to accumulate till it reacts again in an explosive manner, instead of being acted on continuously as long as the ionic concentration is maintained by the current, is not evident. As has already been indicated by one of us⁵ in connection with the activity of certain reflex centers, this

³² Loc. cit., 227.

³³ Garten: Loc. cit., 557.

³⁴ See Howell: Text Book of Physiology, Fourth Edition, 1911, 91; Schafer: Text Book of Physiology, 1900, ii, 510.

³⁵ Forbes: Proc. Roy. Soc. B., 1912, lxxxv, 298.

tendency to rhythmic response denotes some peculiarity in the relation between the substances involved in the processes of excitation, response and recovery not covered by the conception of an unstable substance reacting with ions in accordance with the mass law.

At all events, it is a salient fact in connection with excitable tissues that they may respond to continuous stimulation discontinuously, i.e., with a succession of separate propagated disturbances. Expressing it in terms of Hill's hypothesis, if when the unstable substance is renewed the effective ions are still maintained at a supernormal concentration by the current flow, a fresh disturbance will be initiated. The bearing of this on the effect of the powerful induction shocks which we have been examining is that if the ionic disturbance be great enough and the shock persist long enough with an intensity sufficient to maintain adequately the ionic concentration, the refractory period may end (i.e., the unstable substance be renewed) in time for a second disturbance to be initiated. Erlanger and Garrey³⁶ have shown that induction shocks persist longer than is frequently supposed, and as outlined above it is only necessary that when the refractory period is over, the shock shall not have declined below the intensity required to maintain an effective concentration of ions in order that a second impulse shall be evoked. Judging by the refractory period shown by Adrian and Lucas³⁷ for amphibian nerve, and the temperature coefficient of the same shown by Gotch and Burch³⁸ a powerful break shock may well maintain a local excitatory process long enough to initiate two or even three impulses in a mammalian nerve, even though the local excitatory process outlasts the exciting current by a negligibly short time.

The fact that some of our electrical records produced with powerful shocks show only such deformation as we should expect to result from a second impulse, taken in connection with the theoretic considerations just discussed, makes it seem not improbable that a second impulse may result from shocks of great intensity. In this connection it may be of great significance that make shocks have been found by Erlanger and Garrey to subside far more gradually than break shocks. For in our records, as has already been noted, the deformation is far more marked in the case of make shocks than in that of break shocks under similar conditions, although the break shocks are of far greater stimulating intensity. The explanation may be that break shocks, intense

³⁶ Erlanger and Garrey: *This Journal*, 1914, xxxv, 403.

³⁷ Adrian and Lucas: *Journ. Physiol.*, 1912, xlv, 114.

³⁸ Gotch and Burch: *Journ. Physiol.*, 1899, xxiv, 416.

as they are, subside so quickly that they only produce one or at most two impulses, while the make shocks, persisting longer, may inaugurate a series of impulses comparable to the closing tetanus.²⁹

We do not feel that this view can be confirmed with the data at hand, nor do we believe that the series of impulses can explain all the peculiarities of our more complex records not covered by the transient current already discussed. Still, we feel that these suggestions may constitute a step toward interpreting these rather baffling observations.

We have discussed the deformations at some length (1) because it seems important to emphasize the possible confusion in interpreting action current records due to the entrance of an "artefact," (2) because the electrical disturbance which causes the artefact in the record may have some physiological import depending on the occurrence of stimulation some distance from the physical electrodes, and (3) because the doubling or compounding of the stimulus, which we consider a likely consequence of powerful shocks, has probably an important bearing on the reflex responses, as will later appear.

In dealing with such powerful shocks as those we have been discussing, which produce deformed electrical records, and which may reasonably be supposed to evoke a succession of two or more impulses in the nerve fiber, the problem of evaluating shocks becomes too complex to admit of simple quantitative comparison. Any attempt to compare quantitatively the physiological values of shocks of different contour, as for example the make and break shocks of a given coil, must be made with due regard to its limitations. The rate of subsidence of a shock and of the resultant local excitatory process might well determine the stage of recovery from the first refractory phase at which a second impulse would be initiated. In other words, because, from a comparison which is valid in dealing with thresholds, shocks of different contour would appear to be equal in strength, it does not follow that their physiological effects are inevitably identical; if strong enough to be compound instead of simple stimuli their physiological effects may be quite different.

f. Résumé. Before proceeding to consider the gradation of reflex

²⁹ It should be noted, on the other hand, that we have not recorded many powerful make and break shocks with the secondary wires reversed, and in the cases in which we did (shown in fig. 15) the character of the response was shown to be in the main reversed. Figure 4, B3 shows suggestive results of make and break shocks with wiring reversed from the usual arrangement, illustrating the two types of deformation in their simplest forms.

responses to stimuli of graded intensity it will be advantageous to summarize briefly our findings with responses from the nerve trunk under direct stimulation. Our observations confirm the statement of Gotch that "the response of nerve passes through a series of submaximal stages of increasing value until a maximum is reached." They show that in the case of uninjured mammalian nerves such as we have studied this maximum is reached with break shocks of about 40 Z units, and that with stimuli of several times this value no further increase in the action current is recorded. We find that with more powerful shocks (the requisite strength seems to vary, dependent partly on the distance of the stimulating electrodes from the leads) the galvanometer no longer records simple action currents; the curves show deformation which in some cases appears to result simply from a transient current caused by the connection of the galvanometer with the inductorium and dependent partly upon the distribution of electrostatic capacity in the system (to which the nerve itself must contribute appreciably), which in some cases suggests a succession of nerve impulses, and which in others appear too complex to admit of interpretation with the data at hand.

C. Gradation in the reflex response

a. Electrical. Having dealt with the gradation of action shocks in nerve trunks in response to induction shocks of graded intensity applied directly, and in particular to the gradation of response in the afferent nerve used for reflex stimulation in our experiments, we may proceed to consider the gradation of reflex response resulting from similarly graded shocks. In several experiments measurements were made of the recorded excursions of the string obtained from monophasic action currents in the motor nerve resulting from the usual reflex stimulation. It has been noted in our preceding paper⁴⁰ that even with stimuli of constant strength and though a rest is allowed before each observation, the magnitude of the reflex electrical response is not always constant, but that a progressive increase commonly occurs, and in addition irregularities without apparent cause are often seen.⁴¹ In view of this fact and of the smallness of the reflex responses (no excursions amounting to more than 3 mm.) it was not possible to examine the gradation of reflex responses with anything like the accuracy ob-

⁴⁰ Forbes and Gregg: *Loc. cit.*, 170, 173.

⁴¹ Cf. Sherrington and Sowton: *Journ. Physiol.*, 1915, xlix, 335.

tainable in the case of responses to direct stimulation. We could not determine with certainty whether gradation of reflex response occurred through a given series of stimuli except in those near threshold value. From such measurements as were made it appeared that the response increased from threshold to a maximum which was reached in some preparations at about 25 Z and in some not below 60 Z. After this maximum was reached the responses seemed to remain fairly constant in size and shape even after the stimuli were increased to several hundred Z units. But inasmuch as this was necessarily only a rough estimate, we cannot say with any certainty at what strength of stimulus gradation of response ceases, or indeed whether it really ceases at all.

b. Myographic. Although measurements of the string excursions yielded little information as to the gradation of reflex responses we were able to learn much from observations on the muscular contractions. We made significant observations in many experiments by inspection, and in two we made myographic records of muscular contraction on a smoked drum. In one of these (No. 21) a thread was attached to the ankle and so arranged that flexion of hip and knee should produce a rise in the myograph line. After a series of flexion reflexes had been thus recorded the thread and lever were so arranged as to record the crossed extension reflex, i.e., knee extension in the opposite leg. After a brief series of these the afferent nerve (popliteal) was removed and its action currents were recorded as usual. In the other experiment (No. 29) in which this method was used the muscles of the leg were all paralyzed by section of their nerves except the vasto-crureus and the knee flexor semitendinosus. The tendon of the latter was severed from its insertion and to it was tied a thread connecting it with the myograph lever. In this case the action currents of the afferent nerve were not recorded.

In Experiment 21 there was a progressive diminution in the magnitude of contraction with a given strength of stimulus throughout the experiment, probably due to local impairment of the afferent nerve in the stimulated region.⁴² On this account it was necessary to compare stimuli in alteration. The results of the first part of the experiment in which the flexion reflex was recorded were as follows: A break shock of 68 Z units produced only a small contraction, 82 Z produced a clearly larger one. Satisfactory comparisons were not made between these values and larger ones, but a number of stronger stimuli were compared

⁴² This would mean that stimuli late in the series had less physiological value than appears from the figures assigned them.

with each other. A marked increment occurred in the contraction on passing from 130 Z to 230 Z, 460 Z produced a notable further increment, and 1360 Z produced a marked increment over the latter. It should be noted that none of these flexor contractions obtained from single shocks, even when they amounted to over 2000 Z, were maximal; a series of six or eight make and break shocks of moderate intensity (the breaks being 100 Z) delivered in quick succession, i.e., in the course of one or two seconds, produced almost three times as large an excursion of the myograph lever as the largest produced by a single shock.⁴³

In the series of records of the crossed extension reflex only powerful shocks were used and these were of three values, 1660, 1920 and 2180 Z units. Contractions resulted from single shocks and these were unquestionably graded in magnitude, an increment occurring with each increase in stimulus.

When the afferent nerve used in this experiment was removed and its action currents were studied it showed a graded correlation between response and stimulus of the sort already described as occurring in the majority of experiments (see p. 184, also fig. 1, lowest curve) and taken to signify local impairment of the nerve. An approximate maximum of response was reached at 83 Z, but slight increment persisted well into the hundreds. Moreover with the strongest stimuli the excursions were smaller than are commonly found when nerves in good condition are maximally stimulated. It seems probable that local impairment was such as to render some fibers wholly inexcitable. This state of affairs in the afferent nerve modifies the significance of the results, for instead of a definite limiting maximal value of response reached with stimuli of 40 or 50 Z units we have a persistent gradation of response, and with this we should expect a persistent gradation of reflex response which we might expect to be absent under more normal conditions.

In the other experiment (No. 29) with the myograph, in which the isolated semitendinosus was used as an indicator, the results were somewhat puzzling. Certain irregularities were found, but some facts in regard to gradation stood out clearly. The response to 174 Z showed an increment over the response to 135 Z, and 1420 Z produced a marked increment over 930 Z as is shown in figure 19; 1560 Z produced a further

⁴³ It is interesting to contrast this mechanical summation with the diminution of electrical effect regularly noted in similar series of stimuli in our previous paper, loc. cit., 168.

increment. No observations were made of the action currents in the afferent nerve in this experiment, and this fact prevents direct comparison of reflex with direct stimulation; but the experiment shows in general over how wide a range of intensities in stimuli gradation of response can occur.

c. Inspection. The most significant information was obtained by inspection of muscular movements; and in most cases this was later correlated with the findings from recording the action currents in the afferent nerve. It was readily possible to compare in alternation two or three different intensities of stimulus and to note whether the muscular activity was correspondingly graded.

The type of muscular response to single shocks varied greatly among the different preparations. Sometimes the contractions were only seen

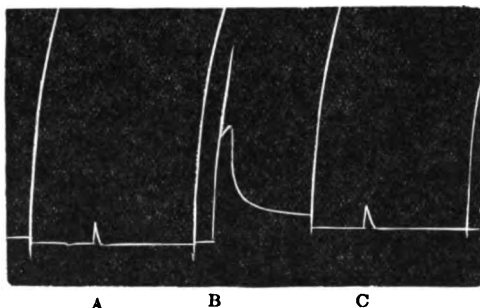


Fig. 19. Contractions of semi-tendinosus muscle in decerebrate preparation. Stimulus applied to peroneal nerve. Experiment 29. Stimuli, single break shocks: A and C, 915 Z; B, 1420 Z. Speed of drum, 5 mm. per second; ordinate lines indicate rests during which the drum was stopped.

in the stimulated leg until shocks of great intensity were used. Sometimes the crossed extension reflex was almost as readily produced as the flexion reflex; in one experiment (No. 15) it followed single shocks of only 6.8 Z units. In the spread of reflex activity to muscle groups more or less remote from the stimulated limb we found the most striking evidence of graded correlation between central effect and stimulus. These muscle groups included

those of all four limbs, the trunk, neck and tail.⁴⁴ It may be stated in a general way that consistent increments in reflex spread were found in the comparison of stimuli of widely varying strength. In some cases increase in the spread of reflexes resulted from increasing the strength of single shocks through the same range over which the response of the nerve trunk directly stimulated shows marked gradation, i.e., from

⁴⁴ Cf. E. L. Porter: This Journal, 1915, xxxvi, 172. It is noteworthy that with single shocks we sometimes evoked forelimb extension, in one preparation with less than 100 Z, whereas Porter never obtained this response in the undrugged animal.

about 10 Z to 40 or 50 Z. In other cases no spread of reflexes occurred with stimuli of less than 100 or 200 Z. The most salient fact was that in practically every experiment gradation in the spread of reflex response followed the grading of stimuli into the hundreds, and when tested, into the thousands of Z units. In most cases large increments of stimulus produced only small increments of response, and in some cases increments of considerable magnitude (e.g., from 93 Z to 120 Z) produced no clearly marked increment of response. On the other hand, in one instance (Experiment 15) an increment in the spread of reflex response appeared with every increment in the following graded series of stimuli: 6.8 Z, 7.4 Z, 17.4 Z, 25 Z, 73 Z, 93 Z, 120 Z, 164 Z, 236 Z, 333 Z, 493 Z. We found regularly that increased spread followed any such increase of stimulus as that from 600 Z to 1300 Z or from 1200 Z to 1700 Z. In one case 3700 Z produced clearly more activity than 2000 Z.

This persistent gradation of reflex response to single shocks is remarkable in view of the fact, noted earlier in the paper, that in the afferent nerve, if uninjured, gradation of response ceases at about 40 Z units, all responses to stronger stimuli being maximal. There appears to be here a striking paradox. The nerve trunk stimulated with a shock of 60 or 100 Z is apparently conducting as great a physiological disturbance as it is capable of, and yet when stimulated with a shock of 200 Z it evokes a greater disturbance in the central nervous system than when stimulated with only 100 Z; with 500 Z a greater response is evoked than with 200 Z, and with 1000 Z a greater response yet. By what additional activity in the afferent nerve trunk is this additional central activity evoked?

Inasmuch as most nerve trunks whose action currents were studied showed gradation of response as far as the responses were capable of valid comparison, probably in consequence of local impairment, it might be supposed that this same local impairment was likewise responsible for the persistent gradation in the spread of reflexes. This may be so in many cases, and yet it is fair to assume that in every case the afferent nerve was far less impaired during reflex stimulation than it was when its action currents were subsequently recorded. For, in the first place, reflex stimulation was always carried out before the recording of action currents, and consequently while the nerve was fresher; and, in the second place, the nerve was always protected during reflex stimulation by the glass tubing of the Sherrington shielded electrodes and kept covered by the muscles which naturally surround it;

in the subsequent removal from the animal's body it was liable to greater impairment than in this first part of the experiment. But even so, we cannot be certain without direct evidence that in the reflex part of the experiment there has not been enough local impairment to render certain fibers inexcitable except to the most powerful shocks, and thus to admit of explanation of the reflex gradation on the same terms as the direct gradation. It is necessary to ascertain whether in a single experiment gradation can occur in the reflex response to stimuli which are subsequently shown by the direct recording of action currents to produce only maximal responses in the stimulated nerve.

Experiment 15, just cited as showing reflex gradation over an exceptionally wide range of stimuli, is of interest in this connection. Local impairment here must have been slight and one of the other progressive factors mentioned as tending to increase the galvanometer excursions must have played a part, for the excursions of the string increased progressively throughout the series of recorded action currents when stimuli of the same strength were repeated. This increase was so marked as to render difficult the comparison of different stimuli. It is nevertheless possible to state that very little true gradation in the action current occurred with stimuli over 30 Z, and that above 60 Z such gradation as may have occurred fell within the limits of observational error, the increase amounting to not more than 2 per cent between 60 Z and 164 Z, above which value the responses showed deformation. It will be recalled that in this experiment gradation of reflex effect was found continuously from 6.8 Z to 493 Z, and in particular at the successive intervals between 73 Z, 93 Z, 120 Z and 164 Z, over which range of stimuli gradation was virtually absent from the responses of the nerve itself.

A clearer and more satisfactory result was obtained from Experiment 21 B. In this case the whole sciatic nerve, examined when still fresh, yielded at 42 Z a clear action current maximum which was sustained to the point of deformation as shown in the uppermost curve of figure 1. Unfortunately, reflex effects of stimuli ranging between 50 and 150 Z were not compared with each other, but a clear increment of response was found with 230 Z over that obtained with 83 Z, and an increment marked each of the following over its predecessor: 230 Z, 330 Z, 467 Z, further increments appearing on passing from 905 to 1160 Z, from 1160 to 1950 Z and from 1690 to 2200 Z. Here, then, is a case in which the afferent nerve was apparently maximally stimulated by every shock of more than 42 Z, and yet successive increments in the disturbance

evoked through it in the central nervous system could be regularly shown to follow every substantial increase in the intensity of the induction shock from this value to over 2000 Z units. The recent experiments of Sherrington and Sowton, already cited, seem to point in the same direction, although their stimuli were not measured in a scale of units which admits of comparison with ours.

It is clear, then, that after induction shocks have been increased in intensity to the point at which, judged by the action currents, they produce maximal stimulation of an afferent nerve trunk, their further increase can in some way so affect the nerve as to cause it to induce in the reflex centers greater and greater activity. In this connection, the observations of Martin and his co-workers should be reviewed. Martin and Lacey⁴⁶ found that in the decerebrate cat induction shocks delivered to any large afferent nerve at rates of from 2 to 60 per second if of moderate intensity produce depressor effects on blood pressure, and that "to obtain pressor reflexes stimuli well in excess of 250 Z units must usually be employed." The average threshold for pressor effects in their series was 280 Z. Martin and Stiles⁴⁶ found that stimulation of the central end of the cut. vagus, under somewhat similar conditions, produced two types of depressor effect, the threshold for the first being about 10 Z, and the threshold for the second being about 200 Z or higher (roughly of the same order as the pressor threshold in the case of the stimulation of other afferent nerves). In a later paper Stiles and Martin⁴⁷ remark of the pressor reflex from stimulating afferent nerves in general that with additional increase of stimulation (above 280 Z) "the elevation of the blood pressure becomes more and more marked through a long range." In these experiments, especially those in which stimuli were applied to the same nerves that we have used, it is probable that examination of the action currents in the stimulated nerves would have shown limiting maximal values in the vicinity of 40 or 50 Z units, inasmuch as the type of electrodes and other significant experimental conditions closely resembled ours. The observations of these workers appear, therefore, to confirm the fact that although an afferent nerve is maximally stimulated (judging from its electrical response) it may not evoke as great a central disturbance as it would if stimulated with more powerful shocks.

A confusing factor might have to be reckoned with when shocks are

⁴⁶ Martin and Lacey: *This Journal*, 1914, xxxiii, 212.

⁴⁶ Martin and Stiles: *This Journal*, 1914, xxxiv, 106.

⁴⁷ Stiles and Martin: *This Journal*, 1915, xxxvii, 95.

applied in rapid succession as was the case in the researches just mentioned, for Adrian and Lucas⁴⁸ have shown that following the refractory period in a stimulated tissue there is first a stage of gradual return to normal conductivity and then a stage of slightly supernormal conductivity. The entire series of changes in amphibian nerve at about 15°C. only lasts at most 0.1 second, and probably much less in mammalian nerves at body temperature. The phase of heightened conductivity could scarcely affect the present case unless there has been profound impairment in the nerve trunk, for we are dealing with supra maximal stimuli; all the fibers are presumably adequately stimulated and conducting impulses with all strengths of stimulus under consideration. The "relative refractory period," during which excitability and conductivity are subnormal, lasts in a nerve trunk at most 0.02 second, too short a time to play a part in the researches we are considering. For Martin and his co-workers found the gradations of response which we have mentioned irrespective of the frequency of stimulation between the limits of 2 to 60 per second. In short, each stimulus must have found the nerve completely recovered from the refractory period following its predecessor, and may, therefore, be considered in the present argument as a single shock. The summation of propagated disturbances which is well known to result in the central nervous system from such frequencies of stimulation as those just mentioned do not concern the issue under discussion.

E. L. Porter,⁴⁹ dealing with reflex effects identical with those which have been the basis of our observations, viz., the spinal limb-reflexes, found that in the spinal cat the threshold stimulus for the crossed-extension reflex varied all the way from 5.8 Z to 300 Z. For stimuli he used single break shocks, and all possible confusion from repetition of stimuli was therefore eliminated. His experimental conditions were closely comparable to ours. The fact stands out from our experiments and from those of others who have reckoned their stimuli quantitatively, that "supramaximal" stimuli are not all equal in their central effects, but that even among them gradation of strength causes corresponding gradation of reflex response.*

⁴⁸ Adrian and Lucas: *Journ. Physiol.*, 1912, xliv, 106, etc.

⁴⁹ E. L. Porter: *Loc. cit.*

*Graham Brown (*Proc. Roy. Soc. B.* 1913, lxxxvii, 142), in obtaining his graded series of reflex contractions used coil distances down to 70 mm. He does not furnish the other data for the evaluation of his stimuli, but it is almost certain that the strongest amounted to several hundred Z units, and would not have yielded simple action current records in the afferent nerve.

D. Proposed explanation of reflex increment

The problem of explaining this fact we have been unable to solve to our satisfaction. Five conceivable explanations have occurred to us, and of these one has been definitely excluded by experiment. They are as follows:

1. Some of the fibers in the afferent nerve, either because of being relatively inaccessible to the stimulating current or of having much higher thresholds than the rest, are only excited by powerful shocks, and are either so few in number or so well insulated by surrounding fibers from the leading-off electrodes that their activity does not add appreciably to the action current as recorded in the galvanometer. We have found no way to test this possibility. If their failure to add appreciably to the recorded disturbance were due to their small number it would be surprising that their meager addition to the total number of impulses could induce in the centers an activity so enormously greater than is evoked by the great majority of the fibers acting without them as is seen to be the case. But if it could be shown that moderate induction shocks only stimulate the surface layers of fibers, leaving the majority of more protected fibers undisturbed, and that the galvanometer records the action currents of only the same surface layers of fibers, then in the stimulation of the inner, more protected fibers with powerful shocks we should have a plausible basis for the gradation of reflex response as we have seen it. Against the probability of this explanation we may mention the fact that we have regularly placed the stimulating electrodes obliquely on opposite sides of the nerve. Thus it is probable that the stimulating current has attained, even with moderate shocks, an effective density in the central as well as the superficial fibers.

2. It may be that the electrical disturbance is not a true quantitative criterion of functional activity. It is quite conceivable that the action current may reach a limiting maximal value and yet the physiological activity be capable of further intensification which reveals itself only indirectly. Lucas⁵⁰ has pointed out that the relation of the electric response to the propagated disturbance is unknown. Gotch⁵¹ has argued that under certain special conditions a nerve impulse may occur without electrical concomitant. The possibility that the elec-

⁵⁰ Lucas: Proc. Roy. Soc. B, 1912, lxxxv, 502-508.

⁵¹ Gotch and Burch: Journ. Physiol., 1899; xxiv, 426; Gotch: Journ. Physiol., 1902, xxviii, 51, etc.

trical response may be limited in magnitude while the propagated disturbance is not, or at least has a different limit, agrees with Gotch's contention as to their separability. Gotch's conclusion was based, first on the observation (made jointly with Burch)⁵² that when two stimuli were applied with an appropriate time interval to a cooled portion of a nerve, only the first evoked clearly appreciable electrical disturbance in a capillary electrometer connected with the cool part, yet when the instrument was connected with a more remote warmed portion of the nerve two responses were recorded. This evidence was reinforced by his later observation that the electrical disturbance could not be detected in a nerve within 4 mm. of a recent injury, although a propagated disturbance could be evoked in and conducted through the same region.

Lucas⁵³ in 1912 in discussing the relation between action current and propagated disturbance, pointed out that Adrian's observations, then in progress (assuming they proved valid) showed how the observation of Gotch and Burch could be explained without the assumption of any lack of parallelism between action current and nerve impulse. Adrian⁵⁴ has since completed and published these experiments in which he showed that when a propagated disturbance passes through a region of impaired conductivity in which it undergoes a decrement, it will, if not wholly extinguished, regain its full magnitude (as judged by its ability to pass through another region of decrement) on emerging into a normal region. Taking this fact in connection with the phenomena of the relative refractory period, Lucas' explanation of the observation of Gotch and Burch becomes well founded.⁵⁵

In like manner we may explain Gotch's other evidence,⁵⁶ the disappearance of the electrical effect in the neighborhood of an injury. Such a modified region may be one of impaired conductivity similar to that studied by Adrian. An impulse entering such a region might be so reduced as to be too small to produce a visible excursion in the electrometer; yet a disturbance initiated in this region might succeed in passing out of it without complete extinction till it reached the unaffected region where it could regain a normal magnitude. Gotch's observation that the neighborhood of a fresh injury manifests hyperexcitability

⁵² Gotch and Burch: *Loc. cit.*, 422.

⁵³ Lucas: *Loc. cit.*, 505-506.

⁵⁴ Adrian: *Journ. Physiol.*, 1912, xlv, 389.

⁵⁵ Cf. Boruttau: *Pflüger's Arch.*, 1901, lxxxiv, 417-424.

⁵⁶ Gotch: *Loc. cit.*, 54.

may seem to contradict this view of impaired conduction; yet this fact might be accounted for in the following way. We have frequently noted in our experiments that rapid severance of an afferent nerve trunk produces a more profound and persistent central effect than any single induction shock, and that crushing or ligaturé produces a more profound and persistent effect still. The former difference might be explained, if the strongest shocks failed to excite all the fibers, by the fact that section necessarily does excite them all, without the assumption of more than single maximal stimulus to each fiber; but the difference between section and crushing shows that in the latter there is something more than a single stimulus. In severe mechanical injury there appears to be a persistent source of stimulation. The way in which such a sustained stimulus would act will be discussed in a later section; but the bearing of it on the present question is that on the basis of Nernst's theory of excitation⁵⁷ a moderate degree of "local excitatory process" may be supposed to last for some time after an injury such as Gotch inflicted. If this were so, then even though the conductivity, and with it the ease of initiating the propagated disturbance, were notably impaired, hyperexcitability might yet be found.⁵⁸

In view of these considerations and the close parallelism between the electrical disturbance and the "propagated disturbance" shown by Lucas,⁵⁹ we are disinclined to accept the view that functional and electrical disturbances are separable to the extent that great increments in the former can be induced by increasingly powerful single shocks after the latter have reached a limiting maximal value.

3. The third explanation was suggested by some of the observations recorded in the first part of the paper on transient electrical effects. We have shown (p. 189) that an appreciable transient current flows through remote portions of a nerve or other conductor similar in resistance and capacity when any part of it is subjected to a powerful induction shock. In our previous paper we mentioned the fact that even when recording the response in nerve to reflex stimulation a notch in the record denoted the passage of such a transient effect through the string.⁶⁰ These notches were not uncommon, and as might be

⁵⁷ See Hill: *Journ. Physiol.*, 1910, xl, 190.

⁵⁸ For the differentiation of the stages in excitation, see Adrian and Lucas: *Journ. Physiol.*, 1912, xlv, 69.

⁵⁹ Lucas: *Journ. Physiol.*, 1909, xxxix, 207; cf. also Boruttau: *Loc. cit.*, 325, etc.

⁶⁰ Forbes and Gregg: *Loc. cit.*, p. 140; also figure 9.

expected were notably of more frequent occurrence in those few experiments in which, with the high voltage magnet, the distal nerve lead was connected with that end of the string which was put to earth through the magnet core than they were in those in which the reverse wiring was employed.⁶¹ The difference is illustrated in figure 20, (A) being made with the former wiring and (B) with the latter. The direction of the current through the coils of the magnet was also reversed in order that the action current should be recorded by a deflection of the string in the usual direction.

The occurrence of a small excursion of the string at the instant of stimulation in these reflex experiments in which the nerve under observation is separate from that which is stimulated shows that from

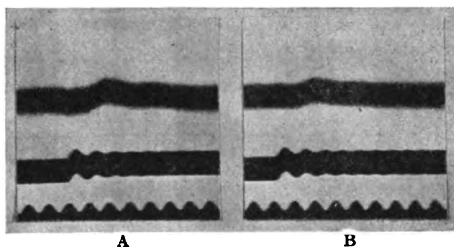


Fig. 20. Experiment 18, reflex responses of peroneal nerve. String D. High-voltage magnet coil. (See text.) Stimulus, 40 Z in each. Cf. previous paper, figure 10, 2 A, and figure 11, 3 B, taken respectively before and after those shown here from the same preparation under practically the same conditions.

the stimulating electrode an appreciable electrical disturbance spreads not only along the nerve trunk but to some extent throughout the entire connected system of conductors, including the animal's body. In view of this fact, it occurred to us that the great spread of reflex activity to remote centers in the spinal cord resulting from powerful shocks might be due to the direct stimulation of the nerve centers by the transient current. It was an easy matter to test this experimentally,

and in those preparations used for the test the result was so definite that only three or four experiments seemed necessary to settle the point. After a series of responses to various powerful shocks had been observed the afferent nerve was crushed at the hip without disturbance to the electrode contacts; a similar series of shocks was then repeated. In one case the muscles adjacent to the point where the stimulated nerve entered the cover of other tissues responded with slight twitches to the most powerful shocks (2000 Z). These were evidently the result of direct or motor nerve stimulation and not of reflex excitation. In

⁶¹ See p. 190, and footnote 25; also p. 193, and figure 11.

the remaining cases no muscular contractions were produced by shocks of over 2000 Z even when repeated rapidly to induce, if possible, reflex summation. Clearly, such transient electric currents as traversed the body of the animal were inadequate to stimulate the reflex centers directly. This tentative explanation of the spread of reflexes must be abandoned.

4. Another possible explanation might be followed on the principle suggested by the shortened latency of the action currents of nerves with powerful shocks (p. 197). It will be recalled that the latency was appreciably shortened with shocks of about 100 or 200 Z, and practically disappeared with still stronger shocks. We pointed out how such shortening of latency might result from the spread of current along the nerve in sufficient concentration to stimulate some of the fibers at a considerable distance from the point where the stimulating electrodes are applied.⁶² If this were so to a marked degree the impulses would be initiated in the various fibers at various distances from their central terminations, for with some variation in the thresholds of the individual fibers, and with some variation in the current density in different areas of the nerve's cross section there would be a wide distribution of the actual points of stimulation among the fibers. Some would perhaps only be stimulated immediately under the physical kathode, while in others an adequate physiological kathode would be found several centimeters nearer the center. In this way some impulses would have a head start over the others, and the effect in the center would be not a "volley" but a "platoon fire."⁶³ On reaching the central network they would traverse common paths not simultaneously, but in succession, and summation might conceivably result. It is a familiar fact that summation occurs to a marked extent in reflex arcs;⁶⁴ Adrian and Lucas have pointed out that it is a "summation of propagated disturbances."⁶⁵ They have suggested that reflex summation may depend only on the conditions which they have produced artificially in the nerve trunk, namely, a region of decrement (impaired conductivity) and the appropriate timing of successive impulses. They have shown that following the absolute refractory period the excitability and conductivity return gradually to normal, then for a time become supernor-

⁶² Cf. Adrian: *Journ. Physiol.*, 1914, xlvii, 473.

⁶³ Cf. Brucke: *Sitzungsb. d. Wiener Akad.*, 1877; Buytendyk: *Zeitschr. f. Biol.*, 1912, lix, 36.

⁶⁴ See Sherrington: *Integrative Action of the Nervous System*, 1906, 36-38.

⁶⁵ Adrian and Lucas: *Journ. Physiol.*, 1912, xlv, 120.

mal. If the picture they present is a true one for all the synapses in the spinal centers, impulses following each other over a common central path to produce summation must be so timed that succeeding ones shall fall outside the refractory periods following their predecessors and in the supernormal stage of recovery. That the refractory period of any conducting path should be so brief that impulses, separated only by the conduction time of the length of nerve over which the stimulating current might effectively spread, would produce central summation, appears most unlikely.

Sherrington and Sowton⁶⁶ have examined specifically the refractory period of the flexion-reflex in the cat by the method of summed contraction, and found it to lie between 1.4σ and 0.7σ in one case, and between 1.1σ and 0.4σ in another. It is not certain from their evidence whether the seat of the refractory period which they are studying is the reflex center or the afferent nerve trunk. Adrian and Lucas⁶⁷ have shown that amphibian nerve at 15°C . has an absolute refractory period of about 2σ , and a total refractory period, absolute and relative, of about 12σ . In the experiment of Sherrington and Sowton with the afferent nerve at 33°C . these times were undoubtedly much shortened, perhaps five- or six-fold. But even so, the relative refractory period, during which the propagated disturbances are subnormal, probably persisted more than 1σ . Lucas⁶⁸ has shown that such a subnormal disturbance on reaching a "region of decrement," such as he suggests the synapse may be, is apt to be extinguished. In this way a stimulus applied to an afferent nerve during its relative refractory period might fail to augment the reflex muscular contraction, and from its failure we could draw no conclusion as to the refractory period in the synapse. That any part of the central mechanism involved in the spread of reflexes has so brief a refractory period as to admit of summation from the overlapping of arcs in the manner suggested, while conceivable, is so unlikely as to render improbable this explanation of the increments we are considering.

5. Our fifth explanation is suggested by some of the considerations discussed in connection with deformed action current records (p. 203). It will be recalled that some of these gave the appearance of being produced by the passage of two or more impulses instead of one. We discussed theoretical reasons for assuming the possibility that a powerful shock, by maintaining a local excitatory state till the end of the refrac-

⁶⁶ Sherrington and Sowton: *Loc. cit.*, 342.

⁶⁷ Adrian and Lucas: *Loc. cit.*, 114.

⁶⁸ Lucas: *Journ. Physiol.*, 1911, xliii, 72, etc.; *ibid.*, 1913, xlv, 475.

tory period, might initiate a second propagated disturbance; or even, in the case of shocks of long duration, several disturbances.

The possibility of two or more impulses ensuing in each afferent nerve fiber renders the problem of the central effect entirely different from that of a single impulse. If we follow the suggestion of Adrian and Lucas (*vide supra*) and regard the central mechanism as a "region of decrement" we may seek an explanation in terms of the summation of propagated disturbances. If we assume that the end branches, synapses or dendrites, involved in the spread of reflexes which occurs only with powerful shocks, have a briefer refractory period than the fibers in the afferent nerve trunk, then we shall have the necessary conditions for central summation. For a second disturbance traversing the afferent fiber in its relative refractory phase might then arrive at the central structure during its supernormal stage of conductivity, and might break through a resistance which blocked the first.⁶⁹

It is premature to assume more than tentatively that the physiology of the central nervous mechanism can be reduced to such simple terms as the impaired conductivity and stages of recovery manifested by a partial block in a nerve trunk. It may well be that a host of other factors must be reckoned with. We cannot be sure that the effect of a second disturbance on the center will be predictable on the basis of the degree of subnormality of the disturbance in the conducting fiber and the degree of supernormality of conduction in the central structure. But it is eminently possible that in some way the central effect of the arrival of two disturbances is different from that of one, and, furthermore, that the effect of two impulses will be modified as they are made to arrive in more or less close succession. In this way it is quite conceivable that an infinite gradation of central effect may be obtainable by the approximation of the second to the first disturbance. Such approximation would result from an increase in the intensity of the local excitatory process which would lead to the initiation of the second disturbance earlier in the relative refractory period.

In support of the view that temporal sequence of propagated disturbances in an afferent nerve may determine the central effect the following observations may be mentioned. In one of those experiments (No. 29) in which myograph tracings were made from the contracting flexor muscle in reflex contraction, we found that with powerful shocks the responses varied strikingly according to whether make shocks or break shocks were employed. The difference is seen in the

⁶⁹ See Adrian and Lucas: *Loc. cit.*, 109.

records reproduced in figure 21, showing the responses to two break shocks and two make shocks. They were taken in alternation, the coil distance remaining the same throughout. The break shocks amounted to over 2000 Z units. It will be seen that make and break shocks each consistently produced a characteristic sequence of contractions and relaxations, but that the sequences differ strikingly from each other. With these extremely powerful shocks there can be little doubt that all the afferent fibers were stimulated in each case. The difference in the type of the response must, therefore, relate to some difference between make shocks and break shocks. Such a difference can well be accounted for in accordance with the principles outlined above, on the basis of the known differences of contour between make shocks and break shocks. The local excitatory process may, in each case, have

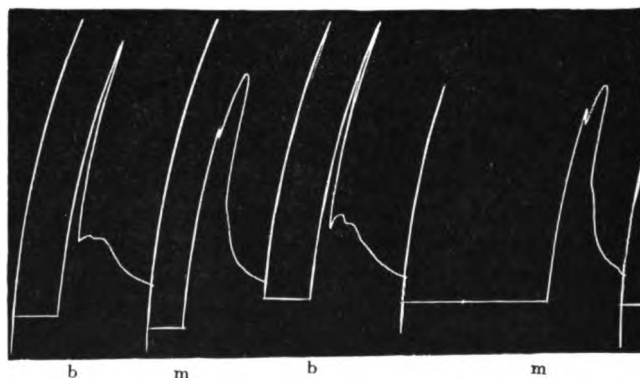


Fig. 21. Reflex contractions of flexor muscle; procedure as in figure 19. Break shocks (b) 2280 Z; make shocks (m) at same coil distance (0 mm). Speed of drum 10 mm. per second; ordinate lines show rests. Cu and Hg key.

been maintained long enough to initiate several propagated disturbances. Inasmuch as the rapidity of rise and decline of the electrical disturbances in the two kinds of shock differs, the state of recovery from the refractory period in which the second disturbance was initiated may well have been different in the two cases, resulting in a different timing of the arrival of the series of disturbances in the center. Upon these differences in timing may rest the observed difference in the motor responses.⁷⁰

⁷⁰ It is significant in this connection to recall the profound alteration of central effect found by Sherrington and Sowton to result from changing the time relations of the stimulating electric current. Proc. Roy. Soc. B., 1911, lxxxiii, 435.

In another experiment (No. 20) at one coil distance make shocks regularly evoked more reflex activity than break shocks which were rated at 1900 Z, although Martin⁷¹ has shown that by comparison of thresholds make shocks are less intense than break shocks by the same ratio at short coils distances as at long ones; and in our experiments the break shocks must have been several times as powerful by the threshold criterion as the corresponding make shocks.

It is also conceivable that powerful shocks, instead of merely producing a persistent "excitatory state" (ionic concentration), may cause some local damaging effect in the nerve structure, thus providing a somewhat persistent source of excitation, much as is the case with mechanical injury or crushing (cf. p. 217). Such persistence of the source of excitation would give rise to a series of successive impulses, and the considerations just mentioned in connection with a purely electrolytic excitatory process of long duration would apply to this case as well.

Of the five explanations we have considered for the observed increments in the spread of reflexes evoked by shocks of increasing strength which appear to have already become supramaximal, as judged by the action currents derived from the afferent nerve, the third has been definitely excluded by experiment; the fourth appears to be unlikely in view of the extremely short refractory period in the synapse which it would demand; the second we regard as improbable in view of the close parallelism shown to exist between the nerve impulse and its electrical manifestation in all researches which give ground for valid conclusions. The first explanation that (moderate shocks only stimulate the surface layers of fibers and only those contribute appreciably to the recorded action current) we regard as somewhat improbable. This consideration may play a part in explaining the facts, but we doubt if it alone can explain the great increase in central disturbance following the most powerful shocks. The fifth explanation appears to us reasonably free from objections and on the whole to be the most probable that we have considered.

It seems to us significant that in most cases the marked increments in reflex activity have appeared with the same intensities of shock which have given rise to the deformations of the action current record already discussed in the previous section. Only in a few instances have we found clear increments of reflex effect between two stimuli subsequently shown both to produce maximal (i.e., ungraded) action

⁷¹ Martin: The Measurement of Induction Shocks, 1912, 101.

currents without deformation. It seems probable that whatever causes deformation in the electrical record is the chief cause of increment in the spread of reflexes.

E. Mechanical stimuli

In two experiments mechanical stimuli were applied to nerves in hopes of securing evidence concerning the relation between afferent impulses and central effect under conditions in which non-physiological electrical disturbances were wholly eliminated. To this end a mechanical stimulating device was arranged by which the sharp edge of a steel spring when released struck the nerve lying on a hard rubber plate; three notches were cut in the trigger whereby the force of the blow could be graded, each notch giving a different tension to the spring. Cutting a nerve with scissors gives a mechanical stimulus to every fiber, but the interpretation of the physiological effect is rendered difficult by the fact that a considerable and variable time must elapse between the beginning and the end of transection, enough time to introduce to a marked extent the possibility of summation in common central paths (cf. p. 219). With the spring device the time of stimulation was rendered as nearly simultaneous in all the fibers as could well be done with a mechanical stimulus. In making comparisons between stimuli with this method it was necessary to move the nerve along and bring a fresh portion under the spring each time, for one stroke generally sufficed to impair the nerve considerably. This necessarily limited closely the number of tests which could be made with a given nerve.

In Experiment 15 one set of comparisons of reflex stimuli made with this device at the three strengths available showed definite gradation in the reflex activity. With greater force the response was more vigorous and persistent and the spread of activity to the fore-limb muscles was more pronounced. By way of control, in Experiment 14 galvanometer records were made of the monophasic action currents of a nerve trunk stimulated directly with the mechanical device. These are reproduced in figure 22 in the order in which they were made, together with one produced by a maximal induction shock for comparison. It will be seen that only with the highest notch was the response maximal. *B*, *C*, *D* and *E* all taken with the middle notch show the degree of constancy obtainable with this method. The nerve was shifted to bring the blow in a fresh region after each stimulus in the series except *B* and *D*. Thus *B* and *C* were taken from successive blows on the same spot, and so were *D* and *E*. The impairment following *B* is ob-

vious. It is also evident that the shift in the point of stimulation didn't suffice to make the response in *D* equal to that in *B*. It will be noted that in *D* and *F* second responses appear in the record. This probably indicates that the spring rebounded and struck the nerve a second time. This possibility renders the method unsatisfactory for quantitative comparisons of reflex responses.

The action current of the motor nerve (peroneal) in response to reflex stimulation through the popliteal nerve with the spring device at the lowest notch was recorded monophasically in Experiment 15. The record is produced in figure 23 together with a record of the reflex response to a break shock made a few minutes later without disturbing the apparatus except to substitute the stimulating electrodes for the spring device. It is interesting to note that the general character and

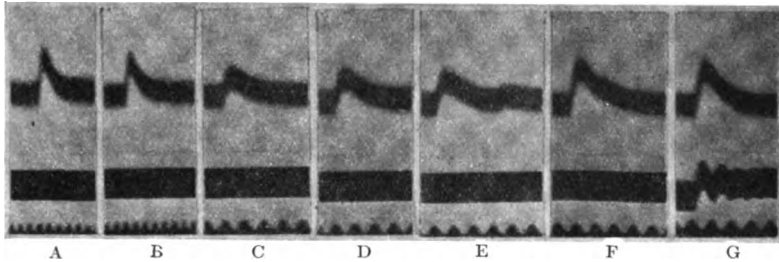


Fig. 22. Responses of peroneal nerve to mechanical stimuli; Experiment 14. Description in text. String D. High-voltage magnet coil with 110-volt current (see legend to figure 15). A, lowest notch; B, C, D and E, middle notch; F, highest notch. Break shock in G for comparison, 59 Z.

time relations of the responses to both reflex and direct stimulation are practically the same with mechanical as with electrical stimuli.

It is evident the spread of reflexes can be induced by a mechanical stimulus so brief that it produces an action current indistinguishable from that evoked by a single induction shock, a fact which argues against the necessity of temporal summation in the causation of such a spread. Beyond this our mechanical stimuli contributed little to the subject under discussion.

III. THEORETICAL IMPLICATIONS OF THE "ALL-OR-NONE" PRINCIPLE

Hitherto we have purposely refrained from accepting Adrian's conclusion that the "all-or-none" principle applies to nerve fiber.⁷² We have tentatively assumed it unproved, and proceeded as if gradation

⁷² Adrian: Journ. Physiol., 1914, xlvii, 460.

of response were possible in the individual fiber. But Adrian's observations cannot be set aside. They lead, so far as we can see, to no other conclusion than his, namely, that the magnitude of response of nerve fiber, as judged by the ability of the disturbance to propagate itself, is independent of the strength of stimulus. They further show⁷³ that except in a region of impaired conductivity where the disturbance is actually undergoing a progressive decrement, this magnitude is independent of the previous history of the disturbance; i.e., on emerging from such a region into one of normal conductivity it regains its normal magnitude. If we grant the validity of these conclusions, and if we assume that the magnitude of the electrical response is a valid criterion

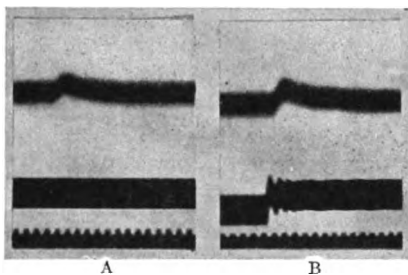


Fig. 23. Experiment 15, reflex responses; description in text. String D. A, mechanical stimulus; B, break shock, 30 Z. Copper point and mercury key.

of the magnitude of the propagated disturbance, then the fact of the limiting maximal value of the action current, which we established experimentally in the first section, becomes an inevitable corollary; for if each fiber responds maximally or not at all then the action current must become maximal when all the fibers are stimulated. Then our preliminary experiments merely show that in a fresh nerve of the size studied all the fibers are stimulated with induction shocks of about 40 or 50 Z units on the

Martin scale, unless indeed a considerable number of fibers in the center of the nerve trunk fail to contribute appreciably to the recorded action current.

We propose now to assume the validity of the "all-or-none" law for the nerve fiber, and to consider the consequences of this assumption in interpreting some of the known facts in the physiology of the central nervous system. In the literature are numerous researches in which gradation of magnitude in the nerve impulse is clearly assumed; the results of many strongly suggest that such gradation is possible. In general, the presence of qualitative changes in reflex response correlated with quantitative changes in the strength of afferent stimulation

⁷³ Adrian: *Journ. Physiol.*, 1912, xlv, 389.

appears hard to reconcile with the "all-or-none" law of the nerve impulse. Why should the involvement of a few more fibers in the nerve trunk change not merely the quantity of reflex response but its entire character? Besides such irradiation of limb reflexes as has been described by Sherrington⁷⁴ and those vasomotor phenomena already referred to as studied by Martin and others, there have been notable qualitative differences in the effects of reflex stimulation of different intensities reported by one of us.⁷⁵ Whereas prolonged stimulation with shocks of one strength produced augmentation of subsequent reflexes, stronger stimulation caused their depression.

It has been quite customary for writers in this field to assume gradation of the nerve impulse as a matter of course. Thus Sherrington in his exposition of the doctrine of "graded synaptic resistance" as the condition of reflex irradiation, written some years before Adrian's researches, put the case as follows:⁷⁶

At each synapse there is a neurone-threshold. At each synapse a small quantity of energy, freed in transmission, acts as a releasing force to a fresh store of energy not along a homogeneous train of conducting material as in a nerve-fiber pure and simple, but across a barrier. . . .

Examining a hypothetical reflex arc *A D* with two synapses, to each of which he assigns a resistance whose numerical value is 2, the afferent, internuncial and motor neurones being designated respectively *A*, *a* and *D*, he goes on to say,

the resistance along *A D* need not, on the numerical values assigned to the synapses sum to the value 4. Yet it is also clear that the threshold for any whole arc cannot be lower than the highest individual threshold in it. Further, the individual thresholds will tend to sum, for an excitation of neurone *A* just sufficient to excite neurone *a* is hardly likely to excite *a* sufficiently to overcome the threshold of synapse *a D*.

It is quite clear that in so far as we deal with impulses following each other after intervals sufficient to obviate confusion from the relative refractory period this doctrine is no longer tenable in the light of Adrian's conclusions. The concept of exciting a neurone "sufficiently to overcome" a synaptic threshold is incompatible with the "all-or-none" principle. Furthermore, if a synapse acts, as Adrian and Lucas have

⁷⁴ Sherrington: *Integrative Action of the Nervous System*, 1906, 150-170.

⁷⁵ Forbes: *Quart. Journ. Exp. Physiol.*, 1912, v, 178, etc.; *This Journal*, 1912, xxxi, 116-120.

⁷⁶ Sherrington: *Op. cit.*, 155.

suggested,⁷⁷ merely as a "region of decrement" comparable to a narcotized stretch of nerve, then the propagated disturbance should, if it were able to pass through the synapse at all, regain its normal size on emerging. If this is the case it renders subject to revision the statement that "the threshold for any whole arc cannot be lower than the highest individual threshold in it." For if a single impulse can pass through the synapses in its path it will induce maximal impulses in every neurone in the chain, and the threshold of the arc must be the threshold of the afferent fiber. Lateral reinforcement from other afferent neurones resulting from the branched arrangement of the central endings might well explain the ability of a volley of impulses in a large number of afferent fibers to excite the central neurones when a smaller number of impulses would fail to do so (cf. p. 219). But the analysis would take a different form from that which assumes gradation of activity in a single fiber.

So far we have dealt with instantaneous stimuli. All the experimental results thus far considered, both in our experiments and in those we have compared with them, have been produced by induction shocks applied to afferent nerves. They all therefore present the same problem discussed in the previous section. We must reconcile the results in each case with the fact that all the effects are evoked by shocks producing full sized impulses in the individual afferent fibers. The differences in the reflex responses must depend either on the number of afferent fibers excited or on some such effect of graded intensity of stimulus as we have considered; i.e., the giving a head start to some impulses with a consequent temporal overlapping in the center (p. 219), or the compounding of excitation in the individual fiber in the manner already analysed (pp. 204 and 221).

But induction shocks applied to nerve trunks constitute a highly artificial laboratory procedure, and it is well to give some consideration to sensory gradation as we know it in normal life. The stimuli by which we are informed of changes in our environment are in a large majority of cases applied not directly to nerve fibers but to special sensory receptors. And in perhaps an equally large majority of cases the stimulus has a duration far exceeding that of the longest induction shock. The importance of these considerations will presently appear.

It is a matter of common experience that we possess the power to distinguish gradations of intensity over a wide range in our ordinary

⁷⁷ Adrian and Lucas: *Journ. Physiol.*, 1912, xliv, 120-122.

sensations. The quantitative estimation of this ability constitutes the well known Weber-Fechner law. It is certain that we recognize extensive gradations of intensity, even in the case of minutely localized stimuli, in the sensations of touch, pain and light. The question arises, how can we detect these gradations if the afferent nerve impulses by which the sensations are transmitted to the central nervous system obey the "all-or-none" law? It might be suggested that the degree of sensation depends entirely on the number of afferent fibers excited by the peripheral stimulus. Thus in the case of tactile stimuli gradually increasing pressure on a sharply localized skin point might give evidence of its increase by transmitting adequate pressure to an increasing number of receptors.

Gradation in visual and auditory sensations cannot be explained in this way. In the emmetropic eye the image of a star however bright cannot stimulate more than a single retinal cone at a time. Yet we clearly recognize several different "magnitudes" of stars as differing from each other appreciably in brightness.* If this were due to the successive excitation of more and more nerve fibers in response to increasing activity in a single retinal cone there would have to be as many fibers in the optic nerve connected with each cone as there are separate intensities of illumination which we can distinguish consciously. Howell⁷⁸ states that in the fovea at least "each cone connects with a single nerve cell and eventually perhaps with a single optic nerve fiber." Barker⁷⁹ states that there are about 1,000,000 cells in the ganglion cell layer of the retina, and about 1,000,000 fibers in each optic nerve. From the size of the retina and the distribution of cones it may justly be estimated that there are at least a million cones in each retina. Thus it is clear that there are not enough optic nerve fibers to admit of sensory gradation by means of graded thresholds in a number of fibers connected with a single cone.

In the case of auditory sensations it is estimated that 4500 resonators must be assumed in order to account for the known discrimination of pitch by the human ear.⁸⁰ If we are to assume that our ability to distinguish different intensities in notes of a given pitch depends on the number of nerve fibers thrown into action by the resonator involved, there must be activated by each resonator as many afferent fibers as

* Cf. Graham Brown: *Loc. cit.*, p. 134.

⁷⁸ Howell: *Text-book of Physiology*, third edition, 1910, 350.

⁷⁹ Barker: *The Nervous System*, 1901, 785.

⁸⁰ Starling: *Principles of Human Physiology*, 1912, 576.

there are distinguishable intensities of sensation at that pitch. It is clear that this is not the case, for at a large majority of audible pitches it is easy to demonstrate with a siren that at least ten intensities can be differentiated; careful experimentation would probably reveal much more delicate gradation. Such discrimination would demand at least 40,000 fibers in the acoustic nerve for transmission, and this is far in excess of the actual number, which has been estimated by histologists at about 14,000.⁸¹

From these elementary facts it follows that even if sensory gradation could conceivably depend, in the case of touch, taste or smell, on the number of afferent fibers excited, no such explanation is tenable for gradation in sight and hearing. Assuming, as we feel we must, the validity of Adrian's conclusion as to the "all-or-none" law, how are we to reconcile it with the obvious sensory gradation unless we assume either that mammalian afferent fibers and amphibian motor fibers, in spite of their similarity in structure and in all objective manifestations of activity, differ fundamentally in the most vital aspects of their physiology, or that the neurofibril and not the fiber is after all the true physiological unit in conduction?

A ready answer to this question is furnished by the researches of Garten, Lucas and Adrian.⁸² The various elements in this answer have already been discussed in detail in previous sections of this paper (pp. 205 and 221); it will suffice to summarize them briefly here.

It must be recalled that the "all-or-none" law as applied to excitable tissues does not imply the impossibility of a propagated disturbance being of other than the standard magnitude under any circumstances. Lucas and Adrian⁸³ have clearly shown that in nerve the relative refractory period, which follows the absolute refractory period, is characterized by lowered excitability and by reduced magnitude of response; a stronger stimulus is required to excite and the resulting response is subnormal. In both respects the tissue returns gradually to normal. When a tissue is stimulated by an electric current a "local excitatory process" is first set up as the direct result of the current, and then a "propagated disturbance" is initiated by the "local excitatory process." The local excitatory process appears to consist in the concentration of certain ions at some limiting membrane within the

⁸¹ See Martin: *The Human Body*, ninth edition, 1910, 199.

⁸² See previous references.

⁸³ Adrian and Lucas: *Loc. cit.*, 114; Adrian: *Journ. Physiol.*, 1913, xlii, 389; Lucas: *ibid.*, 1911, xliii, 77.

tissue;⁸⁴ this process is subject to gradation. The propagated disturbance appears to resemble an explosion in that it uses up all of the immediately available material involved in its production, and leaves the tissue for a time refractory to stimulation. Excitability depends, first, on the ease with which the local process is set up, and, secondly, on the intensity of the local process required to initiate the propagated disturbance. In the gradual return of excitability to normal following a propagated disturbance we are concerned with the second factor, the nexus between local process and propagated disturbance. If the local excitatory process consists, as seems probable, merely in a concentration of ions at some point, we may safely assume that a constant current or a series of rapidly repeated induction shocks will maintain this process to a certain degree. If either constant current or repeated shocks be sufficiently strong and frequent a continuous stimulus will result. As indicated in an earlier section (p. 204), an excitable tissue, when subjected to constant stimulation, responds rhythmically by virtue of the explosive nature of the response and of some property whereby the tissue is permitted to prepare itself for another response while yet protected by its refractory state from stimulation. The Stannius heart, the classical embodiment of the "all-or-none" law, when tetanized exemplifies this principle, and Garten⁸⁵ has shown that similarly a mammalian nerve, stimulated with a constant current of sufficient strength, responds with a series of action currents having a frequency of between 200 and 500 per second at body temperature.

With these facts in mind, we may consider the effect of varying the intensity of stimulation. It is a familiar class room experiment to record the rhythmic contractions of the Stannius heart caused by tetanization with various intensities of induction shock. The gradual return of excitability during the relative refractory period is well illustrated in this way, for with weak stimulation the rhythm of response is slow, while the stronger stimuli the rhythm becomes more rapid; the rate depends upon the stage of recovery at which the particular stimulus becomes adequate.

Adrian⁸⁶ has emphasized the same consideration in reference to nerve trunk as follows:

a strong stimulus will be able to set up a disturbance at a much earlier stage of recovery, when the excitability of the tissue is small. Thus a series of strong stimuli will set up a greater number of disturbances than a series of weak stimuli.

⁸⁴ See Hill: *Journ. Physiol.*, 1910, xl, 190; Lucas: *ibid.*, xl, 225.

⁸⁵ Garten: *Loc. cit.*, 557.

⁸⁶ Adrian: *Journ. Physiol.*, 1913, xlv, 385-386.

In applying this principle to sensory gradation, we are handicapped by knowing little of the laws governing excitation in receptors. We may suppose that their responses to graded stimuli are graded. It may also be that when adequately stimulated they maintain what amounts, as far as the nerve fibers are concerned, to a constant source of stimulation analogous either to a constant current or to a series of stimuli so rapid that their relation to the refractory period in nerve is comparable to that of the tetanizing stimulus to the refractory period of the heart. In absence of more definite knowledge we may fairly assume that this is the case. The fact that sound waves of much lower frequency produce the sensation of a musical note need not interfere with such an assumption, for it is quite conceivable that the resonator serves as a rhythm transformer, passing on to the acoustic nerve endings a virtually constant, or at least a far more rapidly rhythmic stimulus than the sound waves which agitate it. It is not unlikely that after-discharge is a characteristic property of all receptors as it so manifestly is of those concerned with sight. If this were so, then the briefest sensory stimulus, such as a flash of lightning, would be transmuted into a stimulus whose duration would far outlast the refractory period of the nerve fiber to which it was imparted by the receptor.

If the stimulus transmitted by the receptor to the nerve fiber possesses these qualities, gradation, continuity (or virtual continuity) and duration beyond the refractory phase of the nerve, then a basis exists for infinitely graded perception of the strength of sensory stimulation. For as with the Stannius heart, so with the nerve unlimited gradations of frequency in the rhythm of response are possible, each corresponding to a different strength of stimulation. We see no objection to the hypothesis that the brain interprets as evidence of the intensity of peripheral stimulation the rhythm of the nerve impulses by which the sensation is mediated. On this view increasing intensity of sensation would have for its basis increasing frequency of impulses in the afferent nerve fibers.

IV. SUMMARY

1. When a mammalian nerve trunk, such as the sciatic or one of its major branches (popliteal or peroneal) in the cat, is stimulated with single induction shocks of graded intensity, and the resulting action currents are recorded monophasically with the string galvanometer, the magnitude of the electrical response normally increases with in-

creasing stimuli until the latter have reached a value in the neighborhood of 40 Z units; with further increase in strength of stimulus no further increase in response occurs so long as this retains the form typical of a simple action current record, in short there is a limiting maximal value to the action current. When the increase in the strength of induction shock is carried far enough (usually about 200 z in round numbers) the electrical responses no longer appear as simple curves, but show deformation which becomes increasingly marked as the strength of shock is further increased.

If the stimuli be applied to a portion of the nerve which has suffered injury or impairment, the limiting maximal value may be only gradually approached by the response and never quite reached with any stimulus yielding records without deformation, indeed under these circumstances the maximum response obtainable may be considerably smaller than that which results from stimulation in a fresh uninjured portion of the nerve. The failure of such cases to show a definite maximal limit is taken to indicate greatly lowered excitability in many of the nerve fibers, and not to invalidate the general conclusion.

If the action current is a true measure of physiological activity a limiting maximal value of response must of course occur, according to the "all-or-none" principle, as soon as all the fibers are stimulated. In view of this the demonstration of a limiting maximal value of the action current supports the theory that the latter is a true measure of the nerve impulse and in the individual fiber obeys the "all-or-none" law.

2. The causes of deformation in the action current record have been investigated by differential elimination of as many possible contributing factors as we found subject to control.

It is pointed out that the recording instrument need not lie in the path of any part of the current flowing between the stimulating electrodes in order that an electrical disturbance coming directly from the latter shall appear in the record.

Some of the deformed action current records are too complex to be interpreted by consideration of known physical factors. Make shocks in general cause more striking deformation than break shocks.

It is suggested that some at least of the deformations may result from the initiation of a second and even a third propagated disturbance as a result of the "local excitatory process" being maintained with sufficient intensity till after the refractory period following the first. (Cf. Garten.)

3. In examining the question of graded correlation between reflex response and stimulus, in the decerebrate animal, our most significant results have come from inspection. We find that with single shocks the intensity of response in the muscles involved in the flexion reflex and the extensiveness of the spread of reflex activity to remote muscle groups both exhibit striking increments correlated not only with those increments of stimulus which can be shown to produce corresponding increments in the action current of the afferent nerve, but also with increments of stimulus far above the demonstrated maximal value. If we may assume that the electrical response is a true criterion and measure of physiological activity and that activity in all the fibers in the nerve can contribute appreciably to the action current as recorded in the galvanometer, then this long continued series of increments presents an interesting paradox.

Of the conceivable explanations of this phenomenon that have occurred to us we incline to regard as the most probable that a second propagated disturbance, or even a series of them, may be evoked in each afferent nerve fiber by a single shock if sufficiently strong. The arrival of successive impulses in the center may produce a wholly different effect from that of a single impulse, and in the graded rapidity of succession between the impulses there might be the basis of an unlimited gradation in the character or intensity of the reflex response.

4. Mechanical stimuli have been applied to nerve trunks, and action currents have been recorded both from the stimulated nerve and from the motor nerve involved in a reflex response to the stimulus.

5. Our experiments call to mind an apparent incompatibility of the "all-or-none" law (established by Adrian for frog's motor nerve, and presumably applying also to vertebrate nerve fibers in general, both efferent and afferent) and the doctrine of "graded synaptic resistance" which has been used to explain the familiar spread of reflex activity in response to stimuli of increasing strength. The need of revision of some hitherto accepted principles is apparent.

In particular the "all-or-none" law appears incompatible with the delicate gradation of sensory discrimination between different intensities of peripheral stimulation, which is apparent in conscious life. It is shown that in the case of sight and hearing such discrimination cannot be explained by the assumption that an additional afferent nerve fiber is excited at each perceptible increase in intensity of sensory stimulation. It is therefore suggested that inasmuch as excitable tissues respond rhythmically to constant stimulation in consequence of the re-

fractory period, the rhythm of afferent nerve impulses may afford a basis for sensory discrimination of intensity of peripheral stimulation. The work of Lucas and Adrian shows that the recovery of excitability in nerve following its refractory period is such that a sustained stimulus of great intensity would produce a more rapidly rhythmic response than one of less intensity. If the sensory receptors provide to the afferent fibers sustained stimuli of graded intensity, the brain may be apprised of the intensity of the peripheral stimulus by the frequency with which the impulses come to the centers over the afferent nerve fibers.

FURTHER EVIDENCE OF A VASOTONIC AND A VASO-REFLEX MECHANISM

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From the Laboratory of Comparative Physiology in the Harvard Medical School

Received for publication October 28, 1915

I

In the first research on this subject¹ evidence was presented that the general arterial tonus and the vasomotor reflexes are not controlled by the same nerve center.

A second research established a vasotonic and a vasoreflex center.² The method consisted in applying a single reagent to the vasomotor center hitherto believed to control both the vasotonic and the vaso-reflex function. If the center were indeed single, the changes produced by the reagent in the two functions should have been in the same direction. When the reagent increased the reflexes, it should also have increased the tonus. If both tonus and reflexes are the results of the energy of one and the same nerve cell, both functions should have been augmented or depressed as the energy of the cell was augmented or depressed. But curare, the agent employed in those experiments, did not produce this reaction. Only one of the functions was altered. Curare left the tonus unchanged while the depressor and the sciatic vasomotor reflexes were more than doubled. Curare thus separated the vasoreflex from the vasotonic function.

The present investigation confirms that demonstration. It will appear that certain doses of alcohol leave the tonus unchanged while completely suspending the vasomotor reflexes.

¹ W. T. Porter. This journal, 1910, xxvii, 276.

² Ibid., 1915, xxxvi, 418.

II

In figure 1 is shown a typical experiment. On October 22, 1915,³ a membrane manometer was connected to the carotid artery of an etherized rabbit. In the right jugular vein was placed a cannula to which a glass syringe was attached. The left depressor nerve was prepared. On stimulating this nerve with maximal induction currents the normal depressor reflex was obtained, as shown to the left in figure 1; the blood pressure fell from 110 to 75 mm. Hg. Three cubic centimeters of 75 per cent alcohol were now injected into the vein. Three minutes

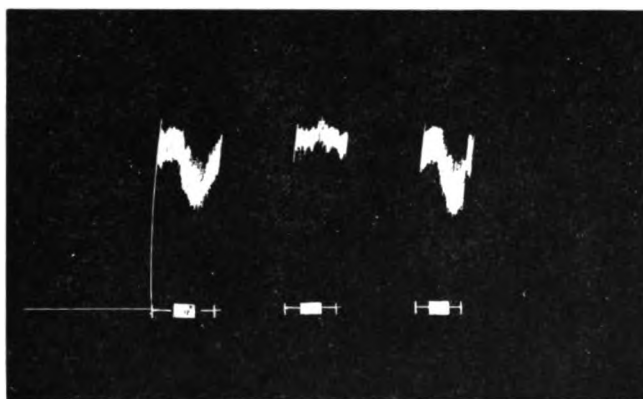


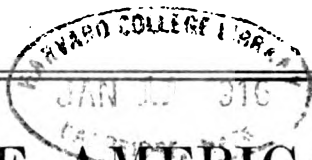
Fig. 1. The effect of alcohol on the arterial tonus and the depressor reflex in the rabbit. The reflex disappears, but soon returns. The tonus remains throughout substantially unchanged.

later, the depressor was again stimulated, but the reflex had disappeared, though the tonus was substantially unchanged. After about five minutes, a third stimulation caused the blood pressure to fall from 108 to 70 mm. Hg. The reflex had returned. The tonus was still practically at the same level.⁴

³ The phenomenon was first observed in November, 1914. A large number of records were obtained in November, December and January. The entire investigation was repeated in March, 1915, and again in October, 1915. In all three series the results were the same.

⁴ It is easy to obtain a lessening of the reflex, but the greatest care must be used if a perfect result is desired. The least mismanagement of the anaesthetic, or the least error in the amount of alcohol, the speed of the injection, or the interval between injection and stimulation will defeat the observation.

Alcohol therefore has an effect opposite to that of curare. With curare, the tonus remains unchanged but the reflex doubles; with alcohol, the tonus remains unchanged but the reflex disappears. Neither phenomenon would be possible, if the vasotonic and the vasoreflex mechanism were identical.



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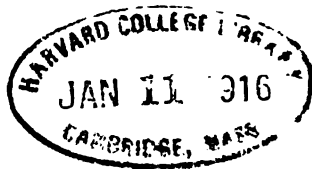
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INITIAL LENGTH—INITIAL TENSION AND TONE OF AURICULAR MUSCLE IN RELATION TO MYO AND CARDIODYNAMICS

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Received for publication November 2, 1915

In previous experiments (1) I showed the beneficial effect of auricular tone on ventricular output, and ascribed this effect to various factors. Among other factors, were increased initial length of ventricular fiber and increased intraventricular tension produced by auricular systole.

A study of the relative importance of these two factors was the object of the present research.

It is well known that the conditions under which a muscle contracts, load, inertia, amount and rate of shortening, affect materially the amount of heat, tension and work developed. Differences of interpretation of fundamental factors concerned in muscular contraction make the literature difficult of review. Only such work as bears directly on this problem will be cited.

Emphasis has been given to both initial length and initial tension as important factors determining the nature of contraction. Blix (2) makes a clear distinction between initial length and initial tension, emphasizing the importance of length of fiber to muscular contraction. If we accept the suggestions of Blix, much of the work done on myodynamics must receive a new interpretation, for it is evident that such factors as load, inertia, initial tension, etc., determine to a great extent the length of fiber at any given moment before and during contraction. But in the work of Schwann (3), Blix (4), Hill (5) and others in which length is regarded as the important factor, initial length of fiber was not varied independently of initial tension: the muscle requiring active

stretching to produce the desired lengthening. Vice versa, initial tension was not increased without an accompanying increase of initial length.

Experiments permitting independent variation of either initial length or initial tension would be valuable in determining the relative importance of each of these factors. The auricle of the turtle through its great and gradual distensibility, and its rhythmic oscillations of tone make such experiments possible.

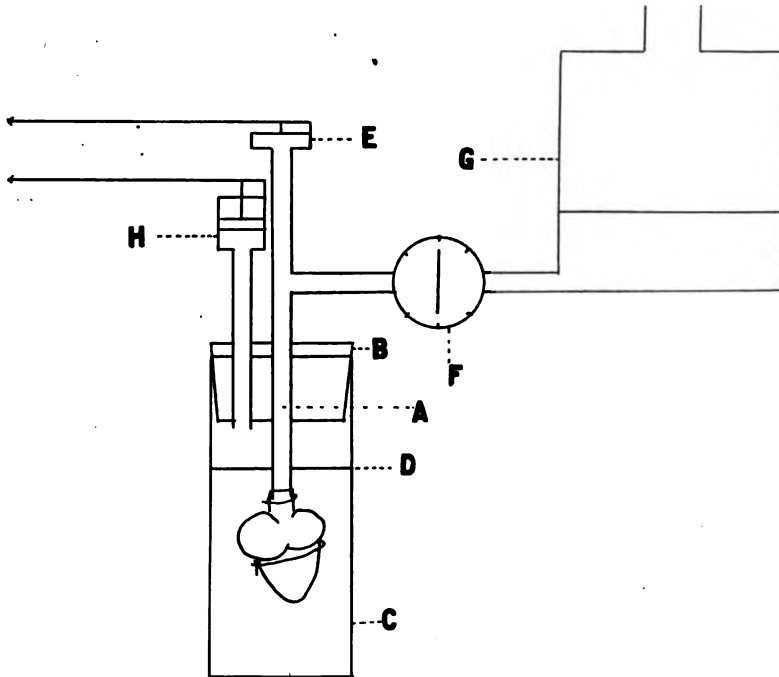


Fig. 1

These auricles show two types of contractions, rapid clonic superimposed upon slow tonic contractions. Bottazzi attributes the slow tonic contractions to the sarcoplasm and the rapid clonic to the fibrillae. Although the results of this research do not definitely prove that such is the case, they point in that direction, and therefore, Bottazzi's suggestions are used in places as a working hypothesis.

Figure 1 shows the method employed. With the heart in situ the left Cuvierian duct, the hepatic vein and the auricular ventricular

junction are ligated. A large cannula is passed through the right Cuvierian duct well into the right auricle and securely tied. The heart is then removed and the cannula connected with tube (a) which passes through the rubber stopper (b). Tube (c) is filled with Ringer's solution to the level (d) and tightly stoppered. The right auricle connects directly with a delicate membrane manometer (e) and indirectly through valve (f) with a large diameter bottle (g). The manometer, tubing and valves are filled with Ringer's solution to the exclusion of air, and the bottle filled to the level shown.

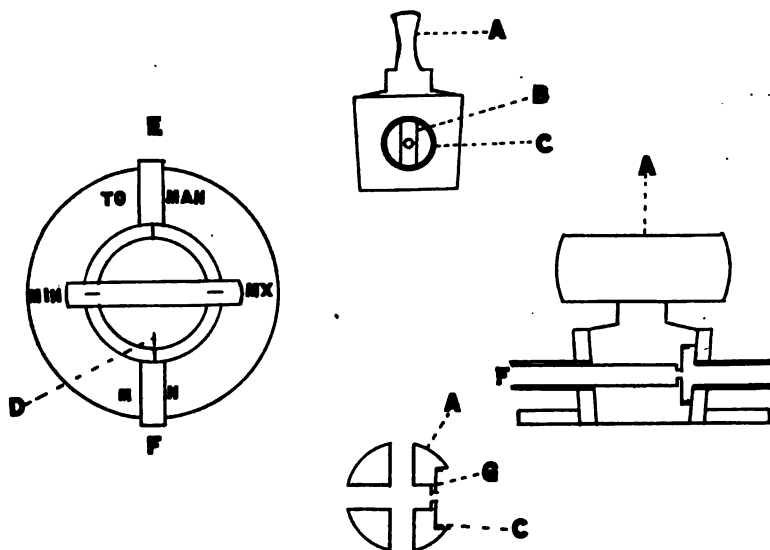


Fig. 2

The auricular filling pressure and the resistance to auricular contraction are largely regulated by varying the height of the bottle. The tension developed by auricular contraction is recorded by manometer (e). With valve (f) open the solution is free to pass to and from the bottle with each contraction and relaxation of the auricles—changing the level (d) in tube (c). These displacements of solution are recorded with the piston recorder (h). Time is marked in seconds and fifths of seconds.

Both isotonic and isometric contractions were recorded. They were obtained by manipulating valve (f). This valve is supplied with a gold beater's skin. Change in the position of the cock therefore

regulates rate and direction of flow through it. Since the valve will serve the more common purpose of a maximum, minimum and mean valve for the registration of pressures it will be described as such. Its construction is shown in figure 2. It consists of three portions, cock, socket, and base. The cock, which is freely removable, has two holes running through it at right angles to each other. One is free and of uniform bore. The other is enlarged at one end, fitted with a centrally perforated platform (*g*). A strip of gold beaters skin is held in place over the center of the platform by a thin brass ring (*c*). The socket is supplied with two tubes (*e*) and (*f*). Tube (*e*) marked "To Man" leads to the manometer. Tube (*f*) connects with the source of pressure. Marks on the socket *mx*, *min*, and *mean* indicate the position of the cock. By rotating the cock to these marks maximum, minimum or mean pressure may be recorded.

The isotonic contractions were not purely isotonic, due to the inertia of the solution, neither were the isometric contractions purely isometric, due to the capacity of the manometer. However, the two types of contractions were distinctly different and had their special values.

With the valve open the solution passes to and from the auricle with each auricular cycle, and so called isotonic contractions are obtained. In these contractions work primarily is performed (see figs. 3, *a* to *b* and 6). The effects of variation of initial length of fiber on these contractions were studied by the use of two methods. (1) Keeping the tension constant and waiting for a spontaneous rhythmic change in length of fiber accompanying auricular tonus oscillation (see fig. 3, *a* to *b*). (2) Sudden increase of auricular filling tension, allowing this rise of tension to progressively lengthen the auricular fiber (see fig. 6). The latter method was also used to determine the effect of tension.

With the valve completely closed the solution cannot move and the auricle contracts isometrically with constant initial and final length. In these contractions tension primarily is developed (see figs. 3, *b* to *c* and 10) and the effect of initial tension may be studied in auricles showing oscillations of tone, for with every tonic contraction and relaxation there is a corresponding rise and fall of tension. The effect of length on isometric contractions may be studied by placing the valve at maximum position, permitting solution to enter, but not to leave the auricle. In such cases a series of isometric contractions with gradually increasing initial length of fiber and constant initial tension are obtained.

In all the records, with the exception of figure 11, the upper tracing

represents volume changes; the middle, tension changes; and the lower time in seconds.

In the volume tracings down stroke represents auricular emptying, up stroke auricular filling. This tracing is an index to volume output, volume, length of fiber, and condition of tone. The upper points indicate the initial volume and initial length of fiber; the lower points, final volume and final length of fiber. In the tension tracing up stroke represents rise of tension, down stroke fall of tension. The upper points of the tension curves represent final tension, the lower, initial or filling tension.

The results of this research were obtained from 27 experiments on the auricle of the turtle. (*Pseudemys elegans*.) In these experiments some of the auricles were free from tonus oscillations while others showed them to varying degrees.

The property of auricular tone alone made this research possible. Naturally a method dependent on auricular tone, giving data concerning the relation of length and tension to muscular contraction, must also throw some light on the mechanism of tone. The nature of auricular tone will therefore be discussed.

RESULTS

1. *Effect of increase of initial length of fiber on isometric contractions of auricles showing marked oscillations of tone* (see figs. 3 and 4).

In figure 3 both isotonic and isometric contractions are recorded: isotonic from *a-b*, isometric from *b-c*. The volume output per beat, the tonus oscillations and length of fiber are represented in the upper tracing. A complete tonus oscillation occurs between points *a* and *b*. At *b* the auricle is at its smallest and is ready to undergo tonic relaxation. Here the valve is turned to maximum position, allowing Ringer's solution to enter but not leave the auricle. A series of isometric contractions follow in which the initial length of fiber gradually increases while the initial tension remains constant. Accompanying the increase in volume (length of fiber), there is progressive increase of final tension.

In figure 4 the effects of increased length of fiber are more marked for a special reason. In that case the auricle is practically empty at the moment the valve is turned while in figure 3 the auricle is fairly well filled. Owing to a more rapid increase in the volume than in the surface of a growing sphere it is evident that the relative as well as the absolute increase in length of fiber is greater in figure 4, than in figure 3.

In addition to the increased final tension note that the duration of maintenance of any given tension also increases with the length of fiber. The importance of this factor in cardiodynamics is strengthened by the fact that the maintenance of the higher tensions is increased relatively more than that of the lower tension.¹

2. *Effect of increase of initial length of fiber on isometric contractions of auricles showing no oscillations of tone, or, at most, weak oscillations.*

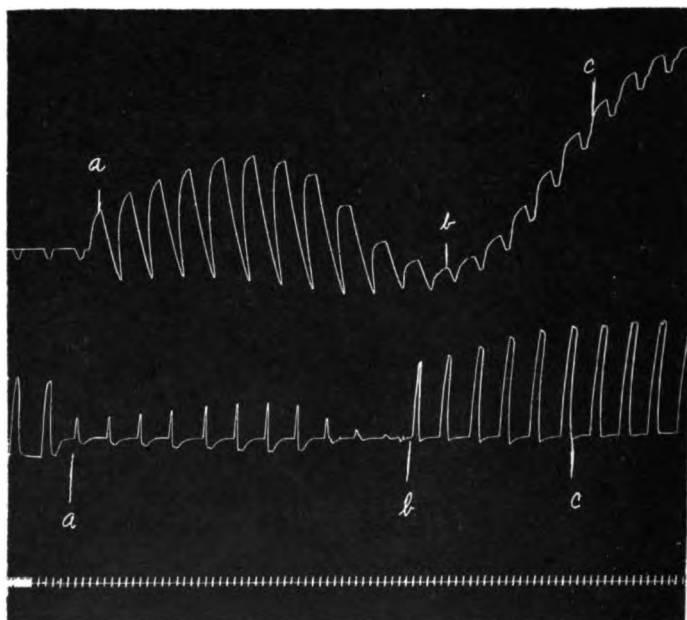


Fig. 3

From the results shown in figures 3 and 4 it might well be argued that the importance of length of fiber was not fully established; that other factors may have entered affecting the efficiency of muscular

¹ A secondary crest appears in some of the figures, e.g., figure 4. This crest is increased in prominence with increased initial length and initial tension. Guenther (20) describes two contractions in heart muscle which might account for tension curves shown in figure 4. In addition the abundant literature (6-19) on the nature of the secondary crest of the contraction curve of striated muscle suggested that the auricular contraction might be composed of a rapid and a slow contraction but the possibility of extraneous liquid oscillations accounting for the second crest does not justify definite conclusions at present.

contraction, for in these records a spontaneous internal condition of the muscle arose permitting for a time the lengthening of the fiber although the filling tension remained constant. This changing internal condition permitting the dilatation of the auricle might also affect the force of contraction, in some unknown way. To determine whether length of the fiber is the factor of prime importance records were taken from auricles which were entirely free from tonus oscillations, or showed them to a small extent only. To obtain a series of isometric contrac-

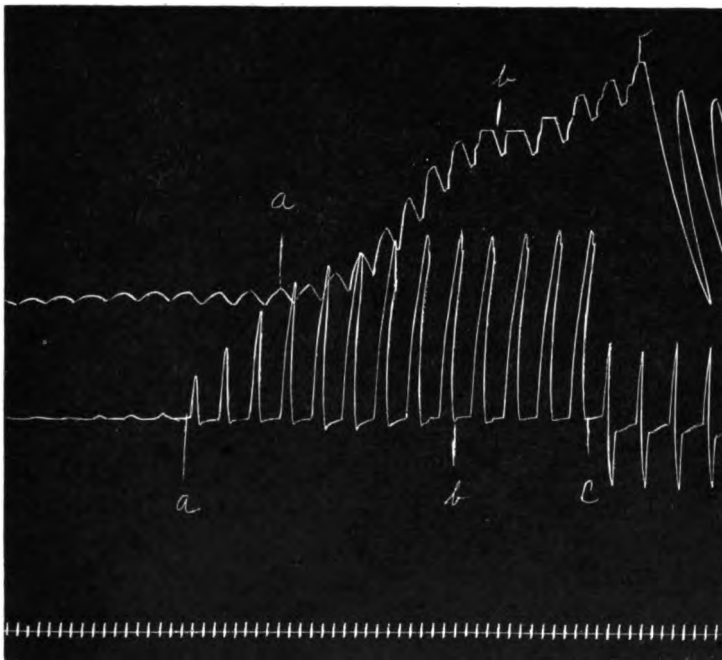


Fig. 4

tions with progressively increasing length of fiber, in such auricles, the valve was placed at maximum position. This usually sufficed to produce a dilatation of the auricle. If it did not, the filling tension was slightly increased, when the desired lengthening invariably followed.

The results obtained show that the increased efficiency of auricular contraction accompanying an increase of length of auricular fiber, is substantially the same—whether the fiber spontaneously increases in

length by some altered internal condition, or is increased in length by some external force. This would indicate that length is the important factor.

According to Bottazzi's (21) theory of tonus oscillation, and Blix's (22) theory of muscular contraction, the varying efficiency accompanying tonus changes can be ascribed to a mechanical factor, a lengthening and shortening of the fibrillae by the contraction and relaxation of the sarcoplasm. Whether this is the only explanation is better studied on auricles contracting under isometric conditions, during a tonus oscillation. Under these conditions, the variation of the important factor—length—is eliminated; the expenditure of energy is concentrated in development of tension alone rather than tension and work and, therefore, variations of auricular energy exchanges are better gauged.

3. *The relation of initial length of fiber to isotonic contractions of auricles showing tonus oscillations* (see figs. 3 and 5).

In figure 3 it will be noticed that the volume output varies directly as the initial volume of the auricle, and that the final tension developed follows an almost parallel course. To interpret the changes in volume output and final tension we must bear in mind a number of factors. The long muscle is more efficient than the short. The volume of the auricle increases as the cube, its surface as the square of the radius. Therefore, the greater the volume of the auricle the greater will be the volume change per unit length of shortening of auricular fiber. Constant initial tension from a mechanical point of view, should have no varying effect on the final tension developed. Inertia of the Ringer's solution affects final tension to an extent dependent on the strength and quickness of contraction and amount of solution to be displaced per unit of time.

The usual relation of length of fiber to solution moved, and tension developed is shown in figure 3.

Occasional exceptions to this rule occurred, and suggest that tonus oscillations are of a more complicated nature than usually considered, and that auricular muscle may strikingly regulate its efficiency by ways other than change in length of fiber. Figure 5 is an example of such an exception. In this record the crests and troughs of the tonus oscillations shown on the upper tracing are marked and set off on the tension tracing below. On the upper tracing two pairs of corresponding points—indicating the same length of fiber—are marked off. Such points, 1 and 2, and, 3 and 4 mark contractions occurring during different phases of tonic oscillation; 3 during the period of tonic relaxation



Fig. 5

C

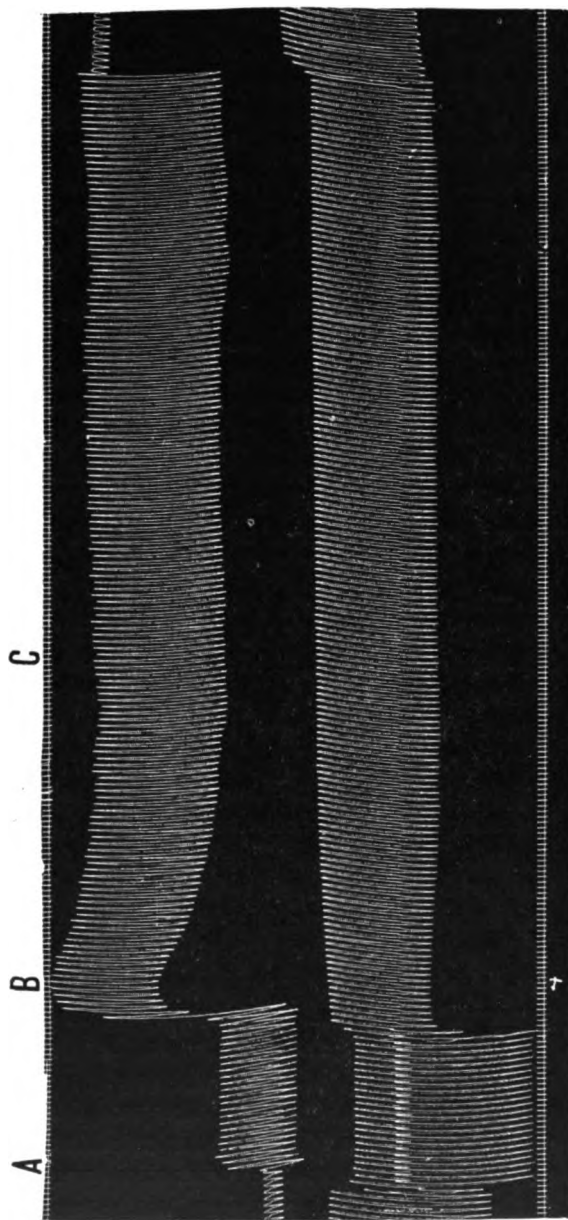


Fig. 6

and 4 during the period of tonic shortening. The amplitude, quickness of contraction, and tension developed are greater at 3 than at 4; this difference occurring although the length of fiber and initial tension are the same in both cases.

1 and 2 mark contractions occurring during the same phase of tonic shortening. The volume and tension changes are considerably different.

Figure 5 shows that changes other than variation of length produce marked differences of efficiency of muscle. Two possibilities, namely changing coordination of contraction, or block are suggested in explanation of this case.

4. *The effect of initial length on isotonic contractions in auricles showing no or small tonus oscillations* (see fig. 6).

If the filling tension of an auricle contracting isotonically is suddenly increased the auricle suddenly grows in volume and from that moment its activities follow a definite course. The sudden large increase of volume gives place to a more gradual increase, and this in turn to a gradual decrease in volume. The relation of volume output and final tension to these changes of initial length of fiber is a special point of interest. In figure 6 the filling tension is increased from 12 mm. to 31 mm. of water. The rapid increase of length of fiber is at once accompanied by an increased volume output and final tension. During the gradual increase in length of fiber the volume output does not increase. After the length of fiber has reached its maximum, final tension and volume output increase, even though the length of fiber decreases.

Since the length of fiber decreases and final tension and output increase, this record indicates a specific enhancing effect of tension on muscular contraction. This effect may be due to increased auricular tone, which usually results from increased filling tension. As will be pointed out tonic force is an important factor in myodynamics.

Experiments showed that the amplitude of volume output and tension developed under isotonic conditions were not reliable indices of the power of the auricle to develop tension under isometric conditions. Therefore, experiments similar to that represented in figure 6 were performed in a slightly modified way (see fig. 7). The initial tension was increased and the auricle allowed for the most part to contract under isotonic conditions. At selected intervals, as the length of fiber decreased, a few isometric contractions were recorded, care being taken to throw in the valve at the end of the period of relaxation. It will be noticed again that as the length of fiber gradually decreases the tension

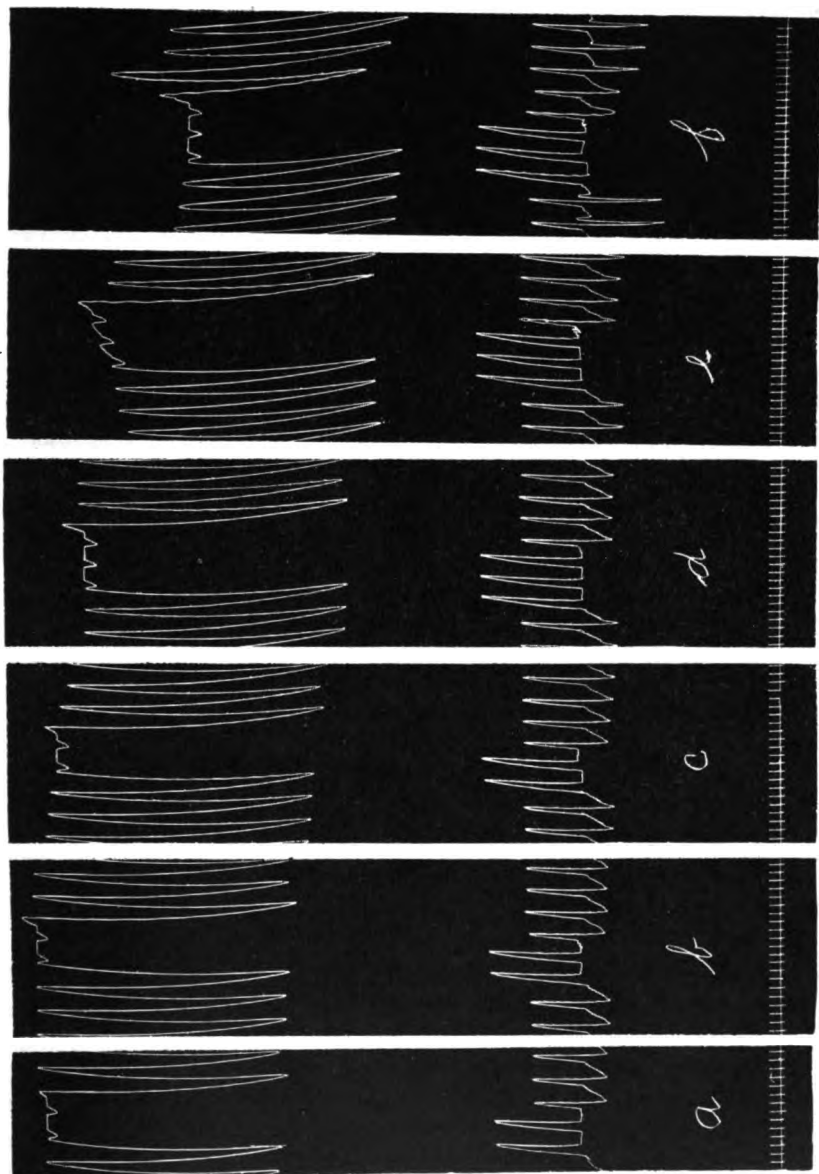


Fig. 7

developed in both isotonic and isometric contractions, progressively increases up to point *F*. The volume output bears a slightly different relation to the changing length of fiber. It reaches its maximum at *E*, then suddenly decreases to a volume output less than that obtaining at *A*. The relation of volume output, initial length of fiber and final tension developed at *F* are of particular interest.

The initial length of fiber at *F* is considerably shorter than at *E* and here the volume of solution moved no longer continues to increase as the initial length decreases. The final tension developed at *F* under isotonic conditions is less than at *E*, but the force of contraction as represented by the isometric contraction is greater. In connection

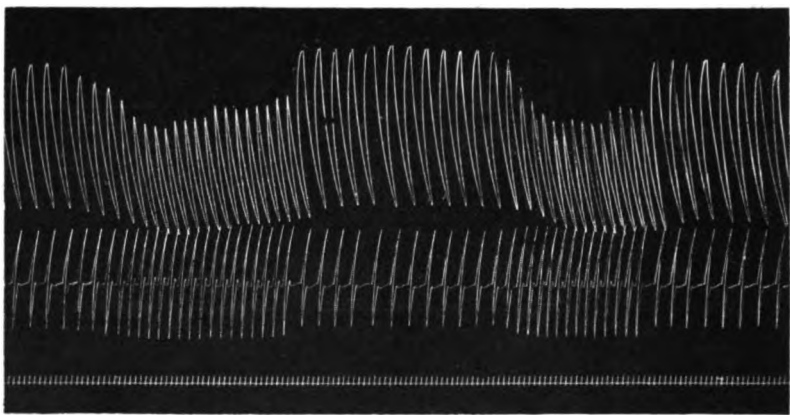


Fig. 8

with these facts it is of interest to note the shape of the volume curves, the sharp angles at the end of relaxation and beginning of contraction from *A* to *E* compared with the rounded curve at *F*, illustrating the importance of tonic force in muscular contraction.

To illustrate again the inverse relation between length of fiber and force of contraction, a chance record different from figures 6 and 7 is described (see fig. 8). In this record groups of frequent contractions alternated with groups of less frequent contractions. When the rapid groups occurred the auricular fiber shortened just as the mammalian ventricular fiber shortens when its sequence is increased. Though the auricular fiber was considerably shortened, the efficiency of the contractions as measured by the tension developed was noticeably increased. Whether the energy liberated was greater or not is

difficult to say, but the record does give evidence that one phase of muscular contraction, namely, quickness, varied inversely as the length of fiber.

5. *The relation of simultaneous change in both length and initial tension to the work performed and tension developed by auricular contraction (see fig. 9).*

Recording tension, length and volume changes under isotonic conditions, starting with high initial tension and lowering this tension by steps, interesting results concerning tonus, final tension and optimum conditions for work are obtained. The results of such an experiment are shown in figure 9 and Table I.

With each diminution of initial tension the length of the auricular fiber diminishes accompanied by changes in final tension and volume output. The volume output first increases (from *a* to *c*). then de-

TABLE I

GROUP OF CONTRACTION	INITIAL TENSION IN MM. OF WATER	FINAL TENSION IN MM. OF WATER	CALCULATED FINAL TENSION IN MM. OF WATER	TENSION DEVELOPED IN MM. OF WATER	RATIO OF DECREASE OF INITIAL LENGTH TO DECREASE OF FINAL LENGTH	VOLUME OUTPUT IN CC.
A.....	81	97	104	16	a/b-7/18	1.00
B.....	60	83	90	23	b/c-8/16	1.55
C.....	30	60	61	30	c/d-19/8	1.74
D.....	17	48	47	31	d/e-16/5	1.42
E.....	10	40		30		1.00

creases (*c* to *e*). Along with these changes a peculiar relationship between the difference in initial lengths and the difference in final lengths of fiber in two adjacent tracings occurred. For instance, between tracing *A* and *B*, and *B* and *C* the difference between the final lengths is great. Between *C* and *D*, and *D* and *E* just the reverse relation obtains. The point of reversal comes just at a time when the effects of tone show on the form of the volume curves. At *A*, and *B*, both contraction and relaxation begin and end abruptly at *C* tonus oscillations are faintly visible. Beyond the point of reversal the end of contraction passes gradually into relaxation. Relaxation is slowed and the base of the relaxation curve is considerably rounded. At this point tonus oscillations appear. The final tension developed falls with every decrease of initial tension but not in the same proportion, as is seen by comparing the calculated tension with the actual tension. The calculated tension of *D* equals the difference between the initial

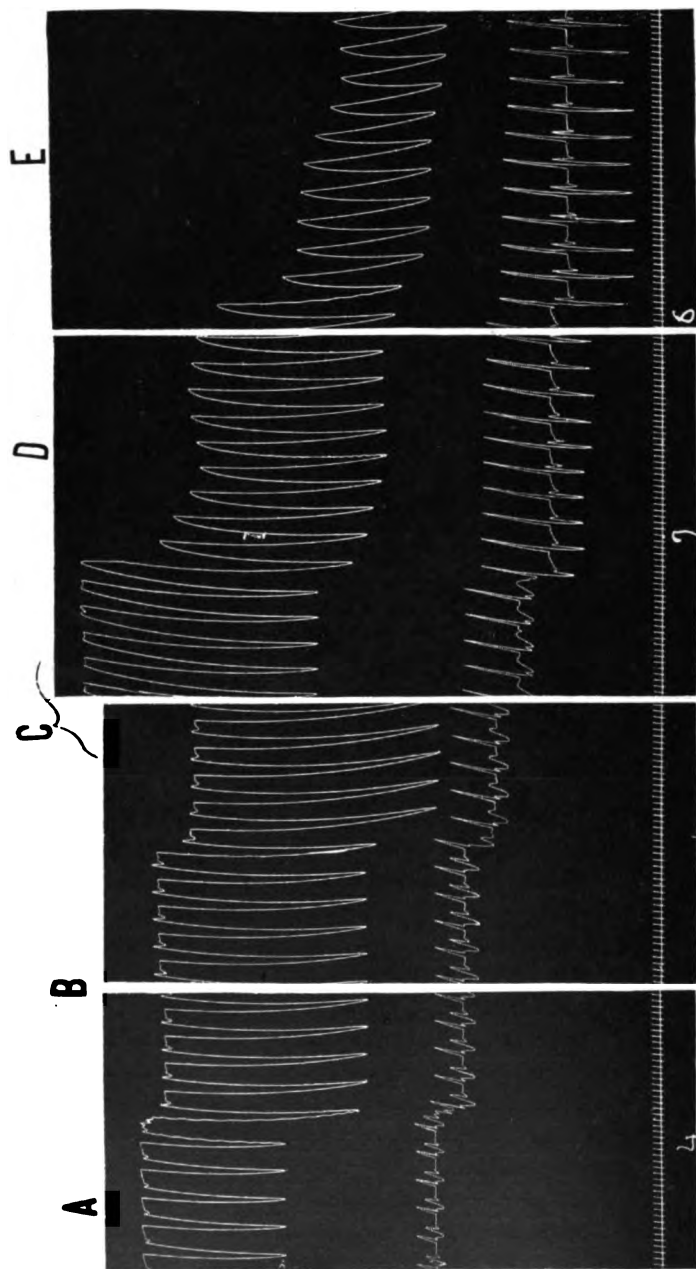


Fig. 9

tension of *D* and *E* plus the developed tension of *E*. For *C* and *D* the calculated tensions correspond closely; for *B* and *A* they fall short. This change like others mentioned occurs at the point of reversal and was ascribed in part to a more or less economic storage of potential energy and resistance to filling tension, these in turn being attributed to the increased relative importance of the tonic force. The volume changes have a somewhat similar explanation: that the volume depends upon a balance of forces—the filling tension working against the elasticity of the muscle (connective tissue and fibrillae), and the changing elastic force of tonus (sarcoplasm). Which group of contractions represents condition favoring maximum efficiency of auricular contraction is difficult to decide. Comparing the differences of tension developed with each contraction and the amount of fluid moved, one would place it at *C*, the point of reversal. At *C* the tonus oscillations are faintly visible. The auricle, therefore, is filled to its maximum without harmful stretching of its fibrillar elements, the sarcoplasmic contractions presumably protecting the fibrillae against harmful stretch. This assumption is made in the light of Bottazzi's theory of tone. In addition, the muscle at *C* is probably more elastic than at *A* and *B* and therefore gives up more perfectly with each contraction its stored potential energy represented by the initial filling tension. Conditions at *D* and *E*, however, are safer for here the muscle is even more elastic. The fibers are shorter and the tonus oscillations more marked. Therefore, among the factors of increased safety may be considered: (1) Greater reserve adaptation at the disposal of the muscle by simple increase in length of fiber; (2) more economic resistance to filling tension; (3) more economic storage of potential energy.

6. Relation of initial tension to isometric auricular contractions of constant initial length of fiber. (See fig. 10.)

If valve *F* is completely closed while the auricle is showing energetic tonus oscillations, the solution cannot move to or from the heart and the length of fiber must necessarily remain constant.

With each tonic contraction and relaxation there is a corresponding rise and fall of initial tension.

The relation of initial to final tension was not constant. But usually, an increase of initial tension led to an increase of final tension. The quantitative relation between the increase of initial and final tension is of special significance. There are indications, which were previously discussed, that initial tension may be stored in the muscle as potential energy. On contraction of the fibrillae this potential energy may be

added to the force generated in the fibrillae. If the increase of final tension is just equal to the increase of initial tension, the effect of initial tension may be purely mechanical and may have no specific effect on the processes underlying contraction. But in figure 10 the increase of final tension is considerably greater than the corresponding increase of initial tension and therefore, cannot be accounted for entirely by the storage of initial tension as potential energy. Some other factor comes into play affecting the processes of contraction proper. What this factor is, is difficult to determine, owing to the fact that the variations of initial tension are the results of tonus oscillations. The interpretation of the records depends largely upon our conception

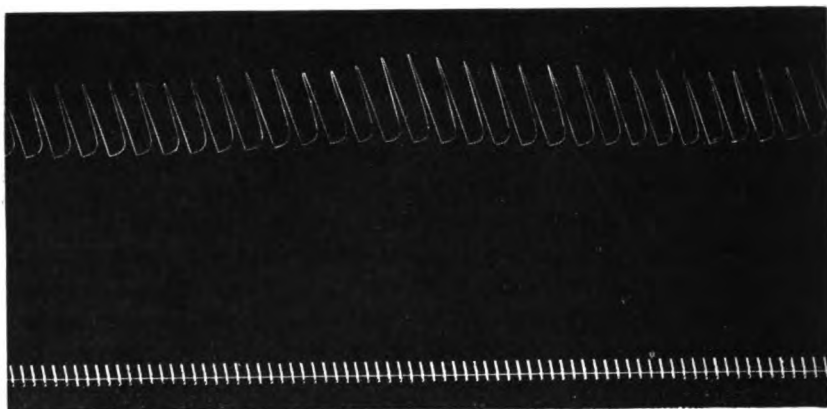


Fig. 10

of the nature of tone. For instance, would the force of contraction be the same in figure 10 if the processes underlying tonus oscillations were not accompanied by oscillations of initial tension? Figure 5 may, in part, answer this question. From 1 to 5 the auricle is contracting isotonicly, from 5 to 7 isometrically with constant initial length of fiber. The relation of length of fiber to strength of contraction in this record was previously discussed. From 6 to 7 the relation of final to initial tension is exactly the reverse of that shown in figure 10. During the period of high initial tension the final tension is actually less than that developed during a period of relatively low initial tension. From 5 to 6 the initial tension remains constant yet the final tension increases. Some factor other than initial tension is responsible for

the fluctuation of final tension, and the question is, do such records prevent us from drawing conclusions as to the effect of initial tension on isometric contraction as seen in figure 10? In answer to this question it can be said that such records as figure 5 are the exceptions; that increase of initial tension is usually accompanied by increase of final tension; that contrary to figure 5, the work and tension developed by an isotonically contracting auricle varies as the length of auricular fiber, whether that length is increased through tonus oscillations or by external force. In figure 5 muscular contractions seem to be affected by oscillations of tone, whether changes of initial tension occur or not. Compare the isotonic record with the isometric. The decreased final tension in both cases invariably accompanies the phase of tonic shortening. The decreased final tension is accompanied by a slower and longer contraction possibly indicating decreased coordination, interference with conduction. If the tonic shortening is always accompanied by the tendency to slower, and longer maintained contraction, through disturbed conduction as suggested for figure 5, the effects of initial tension noted in figure 10 become more significant.

7. *Development of tone.* Oscillations of tone are of frequent occurrence in apparently normal auricles. Some auricles show only small oscillation or are completely free of them. In these auricles the general tone level can be changed by varying the filling tension as shown in figure 6. Here the sequence of length changes, following an increase of initial tension will be recalled: first, a rapid increase; second, a slower increase followed by a decrease in length of fiber. This sequence, as well as the accompanying changes of volume output and final tension were ascribed in part to the development of tone. The influence of tension on tone can be shown in a reverse manner. If the filling tension is decreased in an auricle in which the tonus has been previously increased, the increased tonus shows itself by the auricle contracting down until the cavity is completely obliterated. The auricle remains in this condition for some time, indicating that the tonic force of the auricular muscle is greater than the filling tension; soon the tonus decreases, as evidenced by the gradual increase of auricular volume.

Isotonic contractions can be changed to isometric by closing the valve completely. If the valve is closed at the end of diastole the fiber will be relatively long and the initial auricular tension equal to the filling tension. If the valve is closed at the end of auricular systole, the fiber will be short and initial auricular tension less than the filling tension. In either case the tonus diminishes.

In the first case of isometric contractions, there is continuous tension on the auricular wall, but the active stretch (change in length of fiber) present in the isotonic contraction is missing. This may be the important factor for the development of tone.

8. *Regulation of length of auricular fiber.* An auricle, free from tonus oscillations, contracting isotonically with a constant filling tension maintains a constant volume. If the valve is turned to maximum position the volume of the auricle may or may not change depending upon the condition of tonus (see fig. 11). This figure shows auricular volume changes from two different experiments. If the auricle has a low general tone level (without oscillations) the volume increases promptly on turning the valve to maximum position (fig. 11x). An auricle of higher tone level (also without oscillation) shows no volume change on turning the valve. If such an auricle shows tonus oscillations as in figure 11 y, the volume changes depend upon the moment the valve is turned: if turned at *f*, at the beginning of tonic contraction, no change in volume occurs; if turned at *g*, the volume increases, the curve running parallel to the initial volume curve occurring in an isotonic record (*e-f*). These records bring up the question of the regulation of length of auricular fiber. The two types of behavior on subjection to conditions mentioned indicate that both muscular elements, fibrillae and sarcoplasm, may be factors, varying in importance depending on the relative strength of the tonic force.

Figure 11 x, was obtained from an auricle in which no tonus oscillations were apparent. The tone of the muscle was low. The behavior of the auricle in this case suggests that the fibrillae were the length determining elements, for after isometric conditions are established the auricular fiber cannot shorten; but at end of systole, if the fibrillae have no internal support they may relax and be distended by the filling tension. In the case of figure 11 z, the amount of solution entering the auricle with each diastole is approximately the same for six relaxations, and then gradually diminishes which compares with a distensibility curve of an imperfectly elastic substance.

If the sarcoplasm is the length determining element, owing to the property of maintaining a continued contraction, we should expect, if there are no tonus oscillations and the tonic force is equal to the filling force, no change in volume; if tonus oscillations are present a record corresponding to figure 11 y.

The records seem to indicate that the two elements, fibrillae and sarcoplasm, work together, regulating the gradual and the diastolic

volume changes; the response to distention depending on the relative importance of the two factors. Where the tonic force is weak, as in figure 11 *x*, a constant volume is maintained only while the auricle contracts isotonicly, when contracting isometrically (*c* to *d*) the tonic force is not sufficient to maintain the isotonic volume without the assistance of the resistance to stretching which the isotonicly contracting heart offers during each diastole. If the tonic force is strong

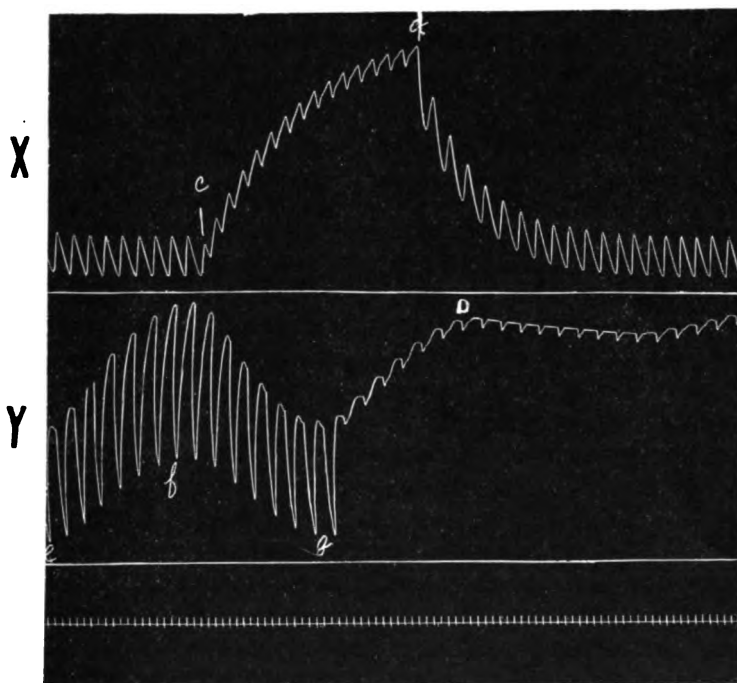


Fig. 11

it is sufficient by its own force to prevent lengthening of the fibers under isometric conditions.

8 B. Relation of rate of contraction to length of fiber. Tonus oscillations may occur in auricles in which the fundamental contractions are suppressed by drugs or eliminated by cutting away the sinus, giving evidence of the relative independence of the two rhythms. It is interesting however, in connection with regulation of length of fiber to note whether there is any relation between rate of contraction and

length of fiber. The relation in the mammalian heart is known: increase in rate producing a decreased length of ventricular fiber. In the turtle's auricle this relation to a certain extent is true, indicating that the rate of relaxation of fibrillae is too slow to maintain the volume obtaining at a slower rate of contraction (see fig. 8). Records were obtained in which groups of frequent contractions alternated with groups of less frequent contraction. In some of these records tonus oscillations occurred. Groups of frequent and less frequent contractions occurred in the various phases of tonic oscillations, but apparently had no effect on the amplitude of the oscillations.

DISCUSSION

The relation of initial length and tension to final tension developed

Both initial length and initial tension have been considered important factors determining the nature of muscular contraction. The relative importance of these factors owing to the difficulty of varying one factor independently of the other, is hard to determine.

Schwann (23) 1873, states that the amount of tension developed by muscular contraction varies directly as the length of the muscle. Blix (24) 1895-1902, finds that the longer the muscle fiber at the moment of excitation, the greater the amount of work, tension and heat developed; the normal extended length of muscle in situ being the most efficient. Employing improved methods of recording heat development, Hill (25) arrives at similar conclusions. In a later study on the frogs sartorius, Evans and Hill (26) find the development of heat and tension to increase on extending the muscle 12 per cent of its normal resting length. While writing this paper the work of Kozawa (27) comes to my attention. Employing methods resembling those used in the present research, he studied the effects of variation of length of the turtle's ventricular fiber in relation to ventricular contraction and found the force of contraction to vary directly as the length of fiber.

In all of the work cited, as far as I am able to determine, increased initial length was accompanied by increased initial tension. Increased initial tension was necessary to produce the desired increase of initial length.

Patterson, Piper and Starling (28) in their paper "On the Regulation of the Heart Beat" show figures in which initial length of ventricular fiber varied, initial intraventricular tension remaining constant. The final tension developed by ventricular systole varied directly as the

length of fiber. These figures they consider "are of special importance since they enable us to differentiate between changes in tension and changes in length as factors determining the amount of energy set free in the contraction of heart muscle." Length of fiber rather than tension was considered the important factor.

Straub (29) dealing with the principles of regulation of heart beat employed the same methods but did not obtain such results. He found initial length and initial tension always to vary together and considers initial tension as the important factor determining the efficiency of ventricular systole.

Since both length of fiber and initial tension have been regarded as important factors in myodynamics the question of clearly differentiating them is important. In view of this fact, and of the fact that the contemporaneous work of Straub and Patterson, Piper, and Starling on the heart are not in agreement, the work reported in this paper was performed.

Turtle's auricular muscle, through its special properties, permits large variations of length of fiber—initial tension remaining constant. Within these limits the ability of auricular muscle to develop tension varies as the length of fiber, agreeing with the conclusions of Schwann, Blix, Hill, and others. This relation holds despite the fact that an increasing surface maintains the increased final tension.

This relation of initial length to final tension suggested to Blix his theory of "chemically active surfaces" as explanatory of his conclusions. According to this theory, the energy exchanges occurring during muscular contraction are dependent on the amount of exposed surface of the active contractile elements. The fibrillae are considered the active elements, their external surface the important surface. Increase in the length of the muscle fiber increases the amount of fibrillar surface exposed and therefore the development of energy. Decrease in length has exactly the opposite effect.

The importance of this conception is evident, and if accepted certain theories of muscular contraction must be changed, for the theory considers the longitudinal surface of the fibrillae as the surface at which the processes responsible for muscular contraction occur. Theories similar to Roaf's (30) in which cross sectional areas are considered important surfaces might require modification.

Tension

The relation of tension to muscular contraction is more difficult to explain. It is known that variations of initial tension or variation of resistance to the muscle during contraction affect materially the nature of contraction. Hill has shown that increase of initial tension and interference with muscular contraction increase the energy exchanges. This increase is in part accounted for by the tendency of both factors to increase the initial length or retard the shortening of the muscle during the first part of the contraction, the period in which the energy exchanges for contraction are determined.

But specific effects of tension other than the influence on length of fiber are indicated in the present research. What is the nature of this influence? Can it be explained on a chemical, physical, or mechanical basis, or must we revert to the theory of secondary excitation?

The effect is partly mechanical. The increased initial tension, whether produced by an external force or by an isometric tonic contraction, is stored as potential energy. When the quick clonic contraction occurs this potential energy is given up and added to the energy developed by the clonic contraction. Hill makes the same suggestion for striated muscle.

The mechanical storage of energy is not sufficient in all cases to explain entirely the effects of initial tension (see fig. 10. a series of isometric contractions of constant initial length, but oscillating initial tension). The final tension changes are greater than the corresponding changes of initial tension. The possibility of the tonic condition of the muscle underlying the variation of initial tension, affecting the final tension was pointed out in connection with figure 5. Other instances of an effect of tension were cited in auricles contracting under isotonic conditions (see fig. 6. The effect of sudden increase of initial tension). From *B-C*, the final tension and the work performed progressively increase though the initial length of fiber decreases. In this instance the rate of auricular rhythm is slightly increased by the change of initial tension. Therefore it might be argued that with the increase in rate, the muscular metabolism may be so altered as to give the Treppe phenomenon. But it seems just as plausible to argue that if the function of automaticity is augmented the function of contractility might at the same time be affected. There is no question but that tonus is increased, and, as has been pointed out, this would increase the efficiency of muscular contraction. If one contractile element (sarcoplasm)

is affected by tension the possibility of the other being influenced cannot be dismissed.

In connection with this suggestion comes the difficulty of knowing definitely in any given condition whether it is the fibrillae or the sarcoplasm which bears the tension. During rest when the tone of the auricular muscle is well developed the sarcoplasm probably bears most of the tension. Sarcoplasm is the nutrient medium of the fibrillae; therefore if the external surface of the fibrillae is the important "chemically active surface," and if the sarcoplasm is altered by change in initial tension as evidenced by the accompanying variations of tonus, the possibility of initial tension having an indirect effect on the fibrillae is indicated.

Nature of Tonus Oscillations

Oscillations of auricular tone were first described by Fano and his associates (31 and 32). They describe two auricular rhythms, a rapid fundamental and a slow undulatory contraction, and conclude that the two rhythms are processes of two distinct elements of the same muscle fiber.

Bottazzi (33) localizes the fundamental contraction in the fibrillae, the tonic contraction in the sarcoplasm, pointing out a direct relation between the intensity of tonus oscillations and the amount of sarcoplasm present.

Botazzi and Grünbaum (34) show the resemblance between sarcoplasmic oscillations of tone and the contraction of smooth muscle, finding the agents or conditions which inhibit or augment auricular tonus oscillations to have an analogous action on smooth muscle.

Bottazzi (35) also points to a direct relation between the prominence of the second crest of contraction of striated muscle and the amount of sarcoplasm present. He finds conditions influencing the prominence of the second crest to have a similar effect on smooth muscle contraction. The absence of rhythmical tonus oscillations in striated muscle he ascribes to the lost property of automaticity.

Mosso (36) however, states that tonus oscillations occur in the respiratory muscles of man.

In striated muscle Grützner (37) allots to the white fibers the function of rapid contraction and to the red fibers slow contraction, which regulates the length of the muscle giving inner support and smoothness to the contraction of the white fibers.

Rosenzweig (38) finding smooth muscle cells in the auricular wall

of the turtle, attributed the tonus oscillations to the smooth muscle and the rapid clonic contraction to the heart muscle proper.

The question relative to the myogenic or neurogenic origin of tonus oscillations is of interest.

Pugliese (39) is of the opinion that the automatic movements of the frog's stomach are initiated by and depend on the integrity of Auerbach's plexus.

Fano states that sections of the turtles auricle entirely free from nervous structures show oscillations of tone. Bottazzi, though believing that sarcoplasm is independently rhythmical, finds the oscillations influenced by vagus or sympathetic stimulation. All workers are agreed that these nerves have an effect. The variation of results of Fano, Bottazzi, Rosenzweig and Oinuma, as Oinuma (40) pointed out, are due to the variations of intensity of tonus oscillations and to the difficulty of isolating for stimulation pure bundles of vagus and sympathetic fibers.

The double innervation of striated muscle described by Perroncito (41), Boeke (42) and others is of interest in this connection, though at present it has not been established for auricular muscle. Perroncito finds in addition to the myelinated fibers, extremely fine nonmyelinated fibers making connection with the motor end plate and other parts of the muscle. No suggestion of the function of these fibers is offered but in a later communication (43) he states that these fibers come from the sympathetic system. Mosso (44) recorded oscillations of tone in the respiratory muscles of man and applied the finding of Perroncito suggesting that striated muscle has a double functional innervation—one for the quick movements, the other for tonic movements; the oscillations of tone being initiated and controlled by cells in the sympathetic nervous system.

Brown's (45) results on gradual compression of nerve support the theory of double functional innervation.

S. de Boer (46) modified Brondgeest's tonus experiment on the frog. Instead of cutting the sciatic plexus he cut the sympathetic fibers going to the plexus and obtained equal decrease of tone. He considers the myelinated fibers as controlling the fibrillae and the nonmyelinated from the sympathetic system as innervating the sarcoplasm. S. de Boer (47) however, was unsuccessful in eliciting only the first contraction of veratrinized muscle on stimulating a nerve bundle free from sympathetic fibers. He, nevertheless, believes that the second contraction is sarcoplasmic, and that the failure to get only the single

quick contractions was due to the stimulation of the sarcoplasm by the muscles' own action current.

The results of this research, while not throwing any definite light on the place of initiation of oscillations of tone and of their nervous control, indicate that these oscillations are a result of sarcoplasmic contraction and relaxation. If the fibrillae easily adapt their length as they seem to do, contraction and relaxation of the sarcoplasm should respectively shorten and lengthen the fibrillae. Then, according to Blix's theory, the sarcoplasm should, by varying the length of the fibrillae, control the efficiency of the muscle. If the efficiency of muscle is altered in such a simple fashion, then it must naturally follow that an increase in length of fiber whether that occurs during a tonus oscillation or is caused by increased filling tension should have substantially the same effect. This is the case.

Though in the main the oscillations of tone may be looked upon as simple contraction and relaxation of sarcoplasm affecting the efficiency of muscular contraction only in a mechanical way, certain results of this research indicate that oscillations of tone influence the muscular efficiency in other ways. Figure 5 is a case in point. The significance of these records has been discussed. Bottazzi's (48) description of two kinds of tonus oscillations is also important in this connection. In one kind, the amplitude of the fundamental contraction remains constant regardless of the change in length of fiber; in the other the amplitude varies directly as the length. The study of Fano and Fayod (49) on the relation of electrical variation in length of fiber is of interest in the same connection.

Figure 5 is important in regard to Rosenzweig's theory of auricular tonus oscillations. It cannot be brought into line with his theory for it is difficult to see how contraction of smooth muscle cells by increasing the initial tension should decrease the final tension.

Development of Tone

Muscular tone is considered a property of normal muscle. But it has been suggested that oscillations of tone in the auricle of the turtle are a pathological manifestation of poorly nourished muscle. Fano states that tonus oscillations occur in normal animals with the circulation intact. It has been my experience that the hearts showing greatest energy also show the largest tonus oscillations and the greatest tendency to develop tone when subjected to conditions which tend to increase tone.

Stretching or increased filling tension was the one stimulus which almost invariably produced an increased auricular tone. The irregularity of rate of tonus oscillation prevented me from determining whether increased tension increased the number of oscillations of tone per unit of time as it did the rapid clonic contractions. One point came out clearly: a definite tension, for each auricle studied produced the largest tonus oscillations. The size of the oscillation is probably dependent on the degree of disturbed balance between force of filling tension and the force of tonic contraction.

An increased filling tension, whether the auricle is contracting isotonically or isometrically almost invariably produces increased tone. Under isotonic conditions the development of tone manifests itself by the shortening of the muscle fiber subsequent to the initial increase in length; under isometric conditions by an increase of intra-auricular initial tension. If stretching stimulates production of tone, does it do so directly as a mechanical stimulus or by electrical variations? Sherrington (50) considers stretching to be the adequate stimulus for nerve endings in such round viscera as the ductus choledocus and ureter. Lewandowsky and Einthoven (51) noted an electrical variation in the vagus on distending the lungs with artificial respiration. Ewald (52) found that stretching the closing muscle of the mussel produced an electrical variation similar to, but in the opposite direction to that produced by normal contraction. These references show that stretching of the auricle may well act as either a mechanical or an electrical stimulus for it has been suggested that a negative variation of a contracting muscle may restimulate that muscle to contraction (53). But in view of the fact that an electrical variation produced by stretching is slow in development and therefore of low stimulating power (54) as compared with the negative variations produced by the rapid contractions and that rapid contractions are not able to maintain the tone of muscle contracting isometrically, it seems that tension exerts its stimulus in another way. It has also been suggested (55) that rising contractile tension, if the sarcoplasm be very irritable, may be of stimulating value. But again, the rising tension occurring during isometric contraction of constant initial length fails to maintain a constant tone.

It seems most plausible that increased tension acts as the adequate stimulus to either the muscle fibers or the nerve endings in them by mechanical stretching. If data of double innervation of auricular muscle were at hand, the latter would seem more probable.

SUMMARY

The relation of initial length, initial tension, and tone of auricular muscle to myo- and cardiodynamics was studied.

Records of auricular tension and auricular volume were made with the auricle contracting under isometric and isotonic conditions.

A method permitting variation of either initial length or initial tension keeping respectively initial tension and initial length constant is described. In connection with this method a maximum, minimum and mean value was devised.

With initial tension constant the strength and duration of contraction varies directly as the length of auricular fiber.

Increase of initial tension while the auricle contracts isotonicly produces a sudden increase in volume which in turn gives way to a gradual decrease. This gradual decrease in volume is accompanied by increasing final tension and volume output, indicating a specific enhancing effect of tension on muscular contraction.

The same enhancing effect of tension is indicated, but not proved in isometric contractions with constant initial length, in which initial tension varies as a result of tonic contraction and relaxation.

The effects of initial tension may be due in part to its storage in the muscle as potential energy and later liberation during contraction.

In addition tension may have a specific effect on the process underlying contraction.

Tonic force is an important factor in auriculo-myodynamics of the turtle.

Both fibrillae and sarcoplasm may be initial length determining elements.

The increased efficiency of auricular contraction accompanying increased length of fiber is substantially the same whether the increased length is artificially produced or occurs spontaneously at the onset of a tonic relaxation—indicating that the varied efficiency of auricular contraction accompanying tonus oscillations is primarily due to a mechanical factor, namely, change of fibrillar length by contraction and relaxation of the sarcoplasm.

There are indications however, that tonus oscillations may affect fibrillar efficiency in other ways.

Tension, or stretching, is the adequate stimulus for the production of tone.

In regard to the relation of auricular systole to ventricular efficiency,

the results show the properties of auricular muscle to be such as to assure good ventricular filling.

Granted that auricular systole increases ventricular filling, the importance of auricular systole is evident.

By increasing the length of ventricular fiber auricular systole increases the strength and duration of ventricular contraction.

By increasing the intraventricular tension it increases the strength of ventricular contraction by adding its own force of contraction as potential energy stored in the ventricular walls.

The presystolic tension arising from auricular systole may have a beneficial effect upon ventricular contraction.

By increasing ventricular filling auricular systole brings about an enhancing surface volume relation. Since the surface of a sphere varies as the square of the radius and the volume as the cube, it follows (if the ventricles are considered roughly spherical) that the larger the ventricular volume the greater the ventricular output per unit shortening of muscle.

When this surface volume relation is considered in connection with the increased efficiency accompanying increased length of fiber and increased initial tension the beneficial effects of auricular systole are all the more evident.

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CONCERNING THE ACTION OF VARIOUS PITUITARY EXTRACTS UPON THE ISOLATED INTESTINAL LOOP

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In their biological reactions the extractives of the adrenal medulla and of the posterior lobe of the hypophysis have many points of resemblance, and when tests are made for minute quantities such as may be present in the circulating blood, their differentiation may be difficult.¹ They both produce a haemodynamic response of some variability, but a pressor action is the dominant feature with a coincident slowing of the pulse rate; they both have a midriatic property on the isolated frog's eye; both are capable of provoking glycosuria and diuresis; both accelerate the coagulation of the blood; both have a specific action on the smooth muscle fibers of the intestine and uterus.

Those who are sufficiently familiar with the two substances can readily distinguish the haemodynamic reactions of one from the other; and adrenalin is more active in producing glycosuria just as pituitary preparations are more potent diuretics. But the most distinctive difference in the actions of the two substances has been supposed to lie in the relaxing effect of adrenalin on the unstriped muscle of the intestine, whereas posterior lobe extracts are commonly believed to uniformly

¹ In Dr. Cushing's monograph on the Pituitary Body and its Disorders (4) the following note occurs: "The similarity of the reactions of adrenalin and posterior lobe extract and the fact that they are both presumably present in the blood stream should make pause those who are accustomed to attribute to adrenalin alone the presence in the blood of a substance which has pressor qualities and which acts on unstriped muscle fiber. It is of interest that J. M. O'Connor (*Ueber Adrenalinbestimmung im Blute*, München. med. Wchnschr. 1911, lviii, 1439) has noted that the action of blood serum is greater on the rabbit's uterus than is the action of an epinephrin solution of the same concentration; and further, that while epinephrin inhibits the intestine, blood serum containing an equal amount of this substance stimulates it. He suggests that some other substance with similar properties must be present. In all likelihood it may prove to be infundibulin."

contract these fibers as well as those of the bladder and uterus. This action on the isolated intestinal loop is regarded by Hoskins (7) as the most sensitive and specific of all epinephrin reactions.

There are some references in the literature which are more or less contradictory to Hoskins's (7) statement that "no other gland shares with the adrenals their depressing effect upon the gut." Dale (5) in 1909 observed that "an isolated loop of intestine, the rhythm and tone of

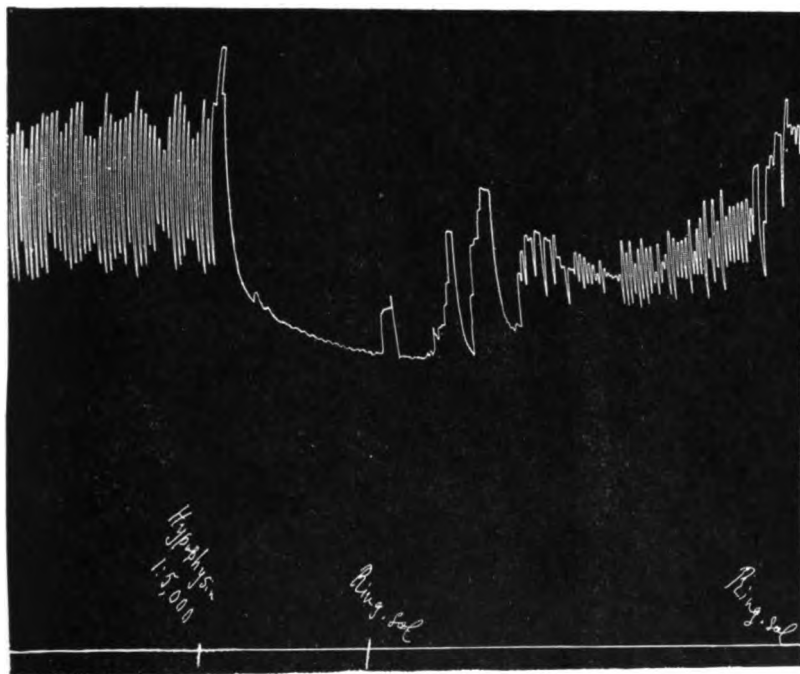


Fig. 1. Relaxing effect on isolated intestinal loop of 1 to 5000 dilution of hypophysin.

which are immediately inhibited by adrenalin, contracts though but feebly when pituitary extract is added to the bath." He supposes therefore that "the inhibition seen under normal conditions of circulation is due to the intense anaemia, which the vasoconstrictor action of the extract produces," and is led to believe "that the characteristic action of the extracts of the posterior lobe of the pituitary body is stimulation of plain muscle fibers." In the same year Foderá and

Pittaü (6) noted that the injections produced defecation, and Bell (2), who studied the influence of intravenous injections of pituitary extract upon different organs, observed strong peristaltic movements in the intestinal tract of rabbits and for this reason recommended the clinical use of pituitary preparations in intestinal stasis. Ott and Scott (8), in 1911 registered peristaltic movements by a balloon inserted in the intestine, and also found that infundibular extracts introduced into

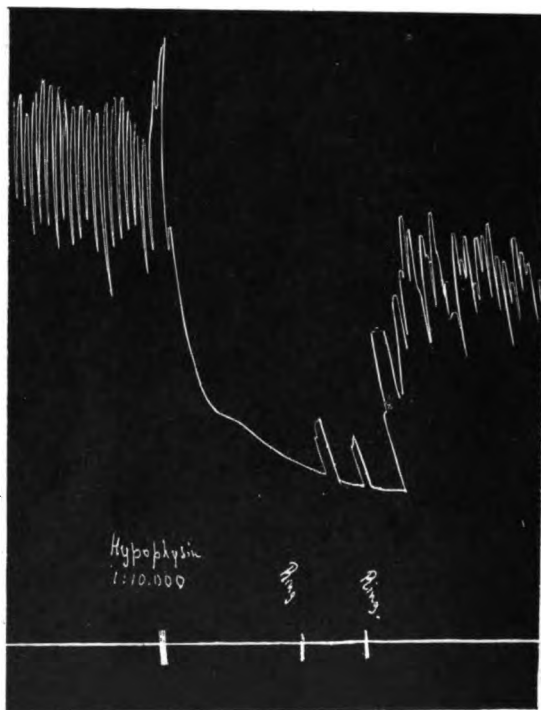


Fig. 2. Relaxing effect of 1 to 10,000 Hypophysin.

the circulation increased the extent of the intestinal contractions. Bayer and Peter (1) alone of all the authors we have consulted noted in 1911 that "puititrin" (Parke, Davis & Co.) and fresh extract of the pituitary body might inhibit the movements and tone of the rabbit's isolated intestinal loop. As Schäfer and Vincent (10) showed to be true of the haemodynamic responses, so also these authors conclude that they are dealing with two substances, one soluble and the other

insoluble in alcohol, the former stimulating, the latter inhibiting, the muscle.

In the course of some experiments undertaken in this laboratory we desired, if possible, to find a delicate reaction for small amounts of posterior lobe substance in the circulating fluids, and under the presumption that the smooth muscle response was the most likely to fulfill our purposes, a series of preliminary tests were made with a variety of

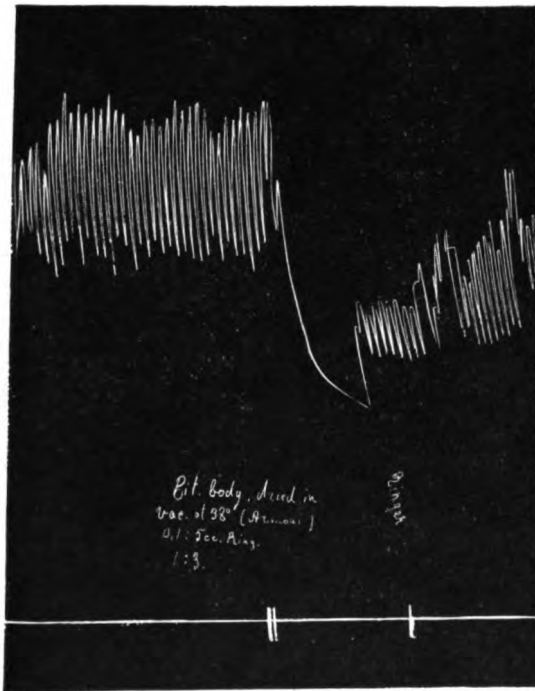


Fig. 3. Relaxing effect of posterior lobe extract dried in vacuo at 38° (Armour).

extracts which were in our possession. In the case of the isolated intestinal loop, it was found, as will be seen, that the effect of these preparations was most variable and inasmuch as reactions supposed to be characteristic of adrenalin were often obtained, this note has been prepared as a warning lest too great reliance be placed on this method as exclusively applicable to adrenalin.

The preparations employed were the following three proprietary fluid preparations: 1, "Pituitary Liquid" (Armour & Co.²); 2, "Pitui-

² Each cc. represents 0.2 gram of the fresh bovine posterior lobe.

tary Extract" ("Schering"); 3, "Hypophysin" (Meister Lucius & Brüning⁴): also the following three dried extracts of posterior lobe which were taken up in Ringer's solution; 4, "Pituitrin A" (Parke, Davis & Co.⁵); 5, dried anterior lobe preparation (Parke, Davis & Co.);

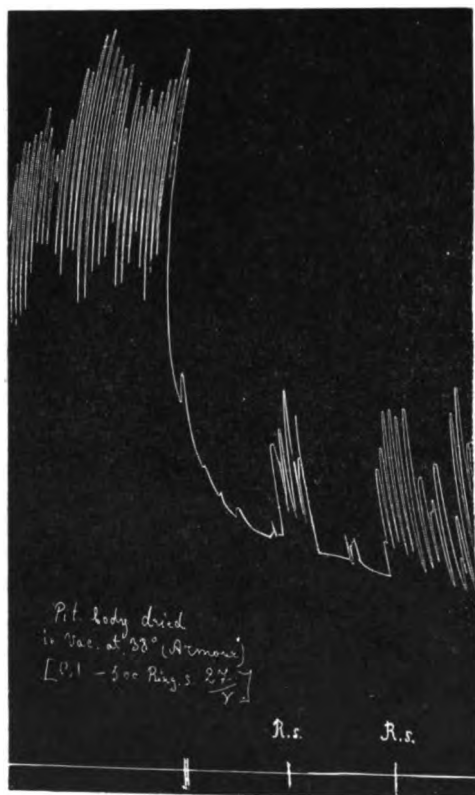


Fig. 4. Reaction from 1 mg. of dried posterior lobe substance extracted with 5 cc. Ringer's solution.

³ Each cc. corresponds to 3 grains of fresh glandular substance of the infundibular lobe.

⁴ This preparation contains the four active substances of the eight substances isolated by Fühner (Deutsch. med. Wchnschr., 1913, No. 11, p. 491) from the posterior lobe.

⁵ This preparation had just been issued by the firm and a sample was placed in our hands. Of the original fluid pituitrin (Parke, Davis & Co.), which had been used by Bayer and Peter, no tests were made as no specimen was in the laboratory at the time. Nor did we test the infundin of Burroughs, Wellcome & Co., or the pituitglandol of the Hoffman-La Roche Co.

6, posterior lobe extract dried in vacuo at 38° (Armour & Co.); 7, dried preparation of (a) Pars anterior, (b) Pars intermedia, and (c) Pars nervosa, the standard preparations of this laboratory from sheep and oxen; and finally, 8, fresh extractives of bovine Pars anterior and Pars nervosa.

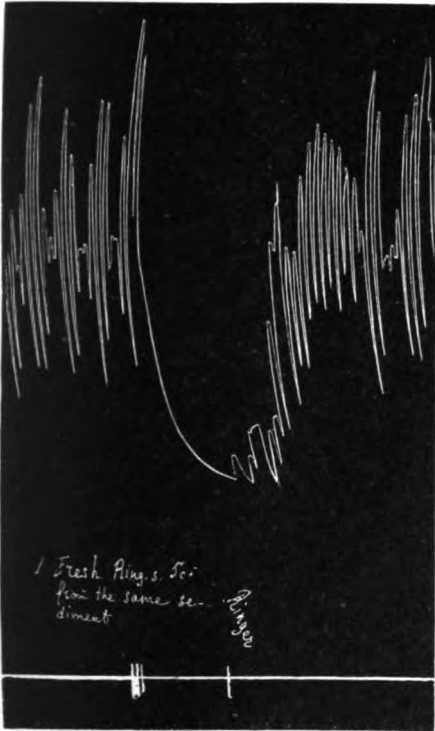


Fig. 5

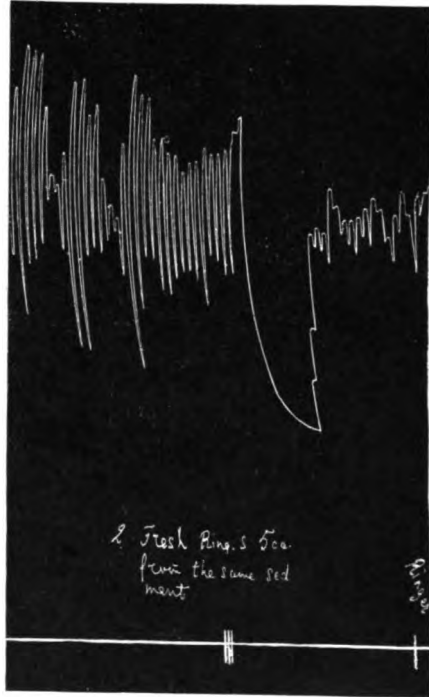


Fig. 6

Fig. 5. The sediment further extracted with 5 cc. Ringer's solution, producing relaxation.

Fig. 6. The third extract of the sediment with 5 cc. Ringer's solution, producing slight relaxation.

The technique described by Hoskins was employed. The segment of intestine from 2 to 2.5 cm. in length was taken from a rabbit anaesthetized by urethan (in 1.5 gram per kilo weight) and suspended in actively oxygenated Ringer's solution at a constant temperature of 38°. The Ringer's solution was drawn off and replaced by the test fluids, kept at an equal temperature by immersion in the outer bath.

For purposes of control, various adrenalin dilutions were employed and they invariably produced the typical relaxation and inhibition of rhythmic contractions described by others.

The various pituitary preparations showed great variability in their response. The preparation which most constantly elicited the reaction supposedly typical of adrenalin, was "Hypophysin" which in dilutions of 1:5000 (fig. 1) and 1:10,000 (fig. 2) gave immediate inhibition of rhythmic movements and sharp relaxation of the isolated loop. The former regular movements and tone were promptly restored on replacing the bath with one of Ringer's solution.

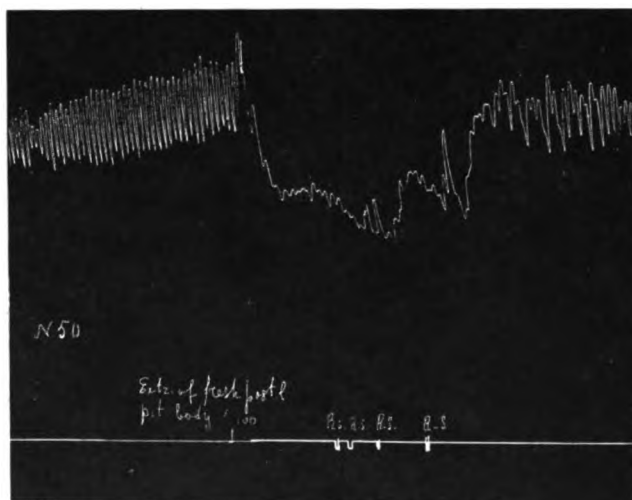


Fig. 7. Relaxing effect of freshly prepared posterior lobe extract 1:100.

The second most potent substance, in this same direction, was the Armour extract of posterior lobe dried in vacuo at 38° (fig. 3). The powdered extract dissolves incompletely in Ringer's solution, but it apparently acts in weak dilutions. The three succeeding records were obtained from solutions made as follows. One mg. of the dried extract was shaken up in 5 cc. of Ringer's solution and after settling, the supernatant fluid was used for the test (fig. 4). To the precipitate another 5 cc. of Ringer's solution was then added with an equally definite response (fig. 5). Again to the second precipitate a third 5 cc. was added with almost as striking a relaxation as the preceding one (fig. 6) and this was repeated several times.

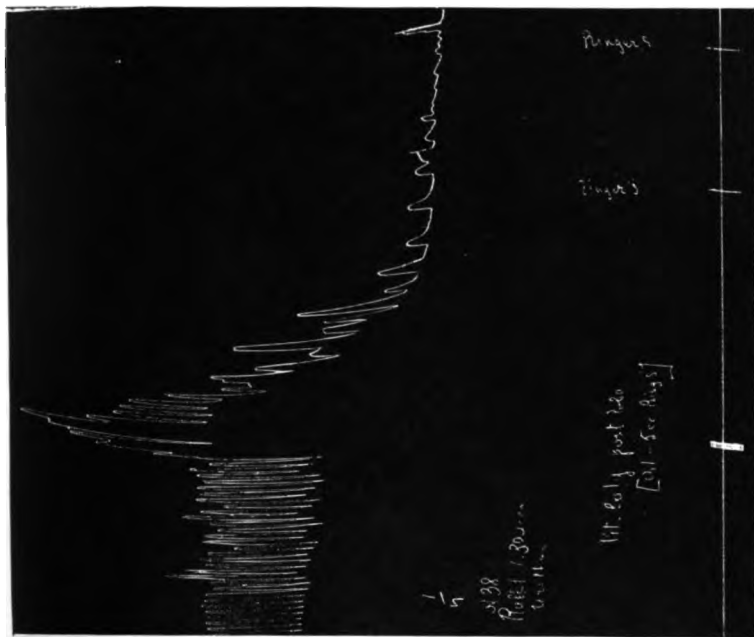


Fig. 8. Momentary relaxation from an extract of fresh posterior lobe 1:250.

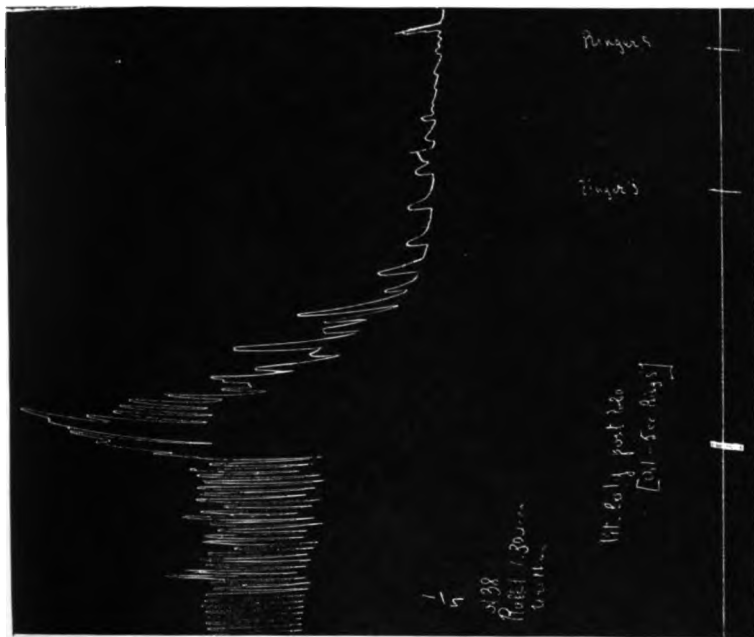


Fig. 9. Response from old dried preparations of posterior lobe substance. Relaxation following contraction.

Freshly prepared posterior lobe extracts made by grinding up the glandular substance with sand were, of our various preparations, the next in their inhibiting tendency on the loop, though some rhythmic contractions might persist during the relaxation (fig. 7) or the total period of relaxation might be brief and incomplete (fig. 8). Some dried laboratory preparations a year old gave some delayed responses occasionally with a primary contraction (fig. 9) and subsequent relax-

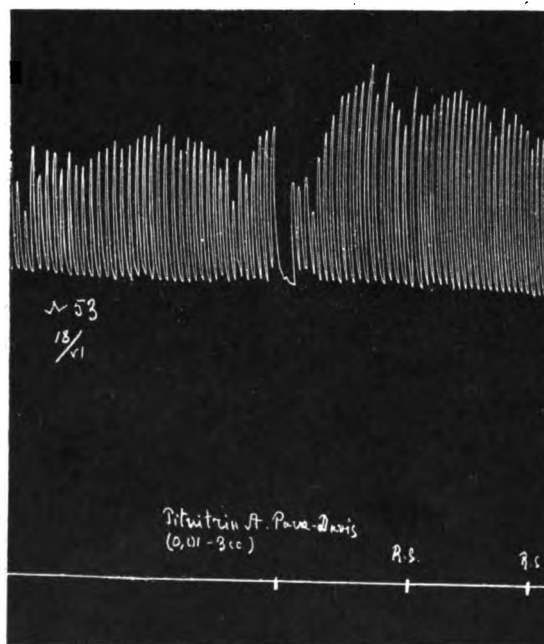


Fig. 10. Slight relaxation from "Pituitrin A" resembling the response (fig. 8) of freshly prepared extract.

ation with incomplete cessation of rhythmicity. "Pituitrin A" also usually gave a suggestive response (fig. 10), though it was incomplete and without definite relaxation, the response resembling that of the fresh posterior lobe extract (fig. 8).

The other posterior lobe preparations (e.g., Armour's "Liquid" and "Schering's" Extract) all gave a more or less outspoken reaction in the reverse direction, namely with an increase of tonicity and activity of peristalsis. Our laboratory preparations of Pars anterior and Pars

intermedia made from the same glands which furnished the posterior lobe extracts were entirely inactive and exerted no influence on the loop.

An effort was made to determine whether in correspondence with their variable action on the intestinal loop, the substances showed any differences as regards their blood pressure and diuretic reactions, but there was nothing conclusive about the responses and even those preparations which had no effect on the isolated loop gave typical haemodynamic responses. Cramer (3) expresses the opinion that the substances which act upon the pupil are not identical with these which stimulate the renal secretion and that the former are not only more delicate but are more easily destroyed, and it is quite probable that in the ordinary preparations we are dealing with more than one basal substance. The wide variability in the physiological activity of commercial pituitary extracts has been emphasized by Roth (9) and though crystalline bodies have been isolated by Houssay, Fühner, Baudouin, Aldrich and others with tentative claims as to their representing the active principle or principles of the gland, they have not been generally accepted as the "active principle" in the sense that epinephrin represents the active principle of the adrenal medulla.

In view of the foregoing observations, it may be stated that there exists in the posterior lobe of the pituitary body some substance which has an action on the isolated intestinal loop resembling the action of adrenalin, a substance moreover which may be other than that which raises blood pressure and causes diuresis. The substance is not constant in the extractives prepared in the usual ways and may indeed be inconstant in the fresh glands from which these substances are prepared. The most definite relaxations were obtained from "Hypophysin" and from Armour's preparation made by drying the extract of the gland in vacuo at body temperature. Its presence or absence in the extracts may depend on the method of preparation and more accurate studies can only be made when this is perfected or when the active principle or principles of the gland become known and are capable of synthesis.

As the matter stands, it may be concluded that certain posterior lobe preparations are capable of producing relaxation of the isolated intestinal loop and of inhibiting its rhythmic contractions, resembling in this respect the extracts of the adrenal medulla.

I desire to express here my gratitude to Professor Cushing for the privilege of working under his guidance.

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ON THE SECRETORY DISCHARGE OF THE PITUITARY BODY
PRODUCED BY STIMULATION OF THE SUPERIOR
CERVICAL SYMPATHETIC GANGLION¹

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In their studies on the autonomic control of the pituitary gland, Weed, Cushing and Jacobson (4) have shown that stimulation of the superior sympathetic ganglion on the neck of animals produced glycosuria even if all the nervous connections between the stimulated part and the abdominal viscera were completely severed. Comparing these experiments with those of direct excitation of the pituitary gland and with the "piqûre glycosurique" of Claude Bernard, both of which also may elicit glycosuria even when all impulses are shut off from the abdominal viscera, the authors came to the conclusion that the glycolytic effect of these excitations, acting upon liver and, possibly, on the adrenal glands with the resultant appearance of sugar in urine, could be explained only through the discharge into the blood stream of hormones of the pituitary gland.

From these investigations it may be concluded that the excitation of Bernard's point on the floor of the fourth ventricle, as well as the stimulation of the superior cervical ganglion, may send impulses which result in the secretory discharge of the pituitary body, through the cervical sympathetic system. This system, as has been histologically demonstrated by Dandy and Goetsch (2), sends terminal fibres to the pituitary body.

These interesting and important facts open a new method of attacking problems concerning the physiology of this organ which is placed in one of the most concealed and protected positions in the body. Up

¹ These experiments were performed during the summer of 1914, but under the difficult circumstances of the present war, the preparation of this paper for publication had to be delayed up to this time. Petrograd, March 23, 1915.

to the present time, the function of the pituitary gland was studied indirectly in two ways, viz., by excluding the organ through its extirpation, or by injecting its extracts into animals. Now, however, by exciting the superior cervical sympathetic ganglion, a structure comparatively easy to approach, we may produce the secretory discharge of the hypophysis directly, and proceed then to observations upon the effect of its hormones under more natural conditions.

The present study may be looked upon as a continuation of the work of Weed, Cushing, and Jacobson, and deals with the effect of the stimulation of the ganglion upon the urinary secretion, a matter which was only touched upon by these authors. Since Schäfer and Herring's (3) classical experiments, diuresis must share with the characteristic blood pressure response as one of the most typical phenomena produced by the injection of extracts of the posterior lobe.

In the technical part of my experiments on the flow of urine I applied Schäfer and Herring's methods. With the narcotized animal on its back, the urethra was exposed by a *sectio alta* and a glass catheter was introduced into the bladder through this incision. If the catheter was carefully inserted, no trouble was experienced in getting a regular flow of drops which were registered on the drum of the kymographion.

After insertion of the catheter, the regions of the two carotid arteries were exposed and in doing so, with least possible manipulations, the two vago-sympathetic trunks on both sides of the neck in their mid-course were divided. Then the tissues in the region of the left superior cervical ganglion were carefully dissected without actually exposing the ganglion itself, in order to avoid any traumatic excitation at this time. After inserting a cannula into the right carotid artery, the blood pressure, as well as the falling drops of urine and the respiration were all recorded.

After defining the normal blood pressure and the rate of urinary flow at this stage, the left cervical ganglion was then finally isolated and stimulated by a faradic current. The moment of the exposure and of excitation of the ganglion were noted on the tracing. Subsequently on disappearance of the primary reaction the excitation was repeated several times with increasing strengths of the current.

Almost all of these observations have been made on cats, for these animals, as shown by Schäfer and Herring, are the most suitable ones for demonstrating hypophyseal diuresis. Moreover, in cats the superior cervical ganglion is situated much lower in the neck than in other animals and is, therefore, more accessible and more quickly exposed;

then again, the stimulation of the ganglion can be made with greater exactitude. Only a few experiments of a preliminary character were carried out on dogs and rabbits.

The animals used for observations were in some cases anaesthetized by means of urethan, administered through a stomach tube in the dosage of 2 grams pro kilo. In other cases the animals were narcotized by the intratracheal insufflation of ether.

My first series of experiments consisted in stimulating the sympathetic ganglion subsequent to a division of the cervical nerves, as related. The results were fairly definite, as may be gathered from the following sufficiently typical protocol:

Experiment No. 7. June 11, 1914.

Cat, weight 3 kgm. Urethan, 6 grams.

Division of both vago-sympathetic trunks in the middle of the neck. Drops of urine issuing at intervals of 12 seconds each. The flow of urine soon after exposure of the superior sympathetic cervical ganglion increased in velocity to a drop every 2 or 3 seconds.

11.54 a.m. Faradic stimulation of the ganglion. Subsequent cessation of flow with a single drop only after an interval of 1 m. 12 seconds. This was followed by a distinct acceleration of drops of urine with about a 1 second interval (fig. 1).

The blood pressure which was at first about 104 mm., after the stimulation of the ganglion showed a short depressor phase to 88 mm. and subsequently rose to 148 mm.

The acceleration of the urine flow, which was often observed in cases such as the above, could not without reservations be ascribed to a pituitary discharge, for in experiments conducted in the above manner, some stimulation might have been transmitted by way of the spinal cord to the abdominal viscera.

Consequently in a subsequent series of experiments I proceeded still further, and not only divided the aforementioned nerves in the neck, but transected the spinal cord as well, this being done at the fourth thoracic segment, i.e., above the level of emergence of the first splanchnics. When acutely performed, this operation commonly produces a decided decrease of blood pressure followed by a prolonged anuria, so that a preliminary aseptic surgical transection of the spinal cord at T4 level was performed and not until full recovery was the observation made of stimulating the sympathetic ganglion.

With this preliminary transection of the spinal cord, I have performed 15 experiments. Out of these, in eight cases the excitation of the sympathetic cervical ganglion resulted in decided diuresis, fully resembling that which follows an intravenous injection of posterior

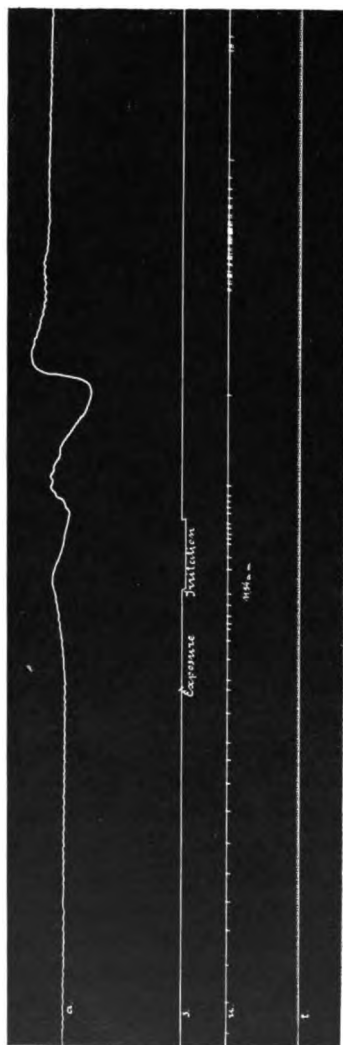


Fig. 1. Exp. N 7. *a*, arterial blood pressure; *s*, signal and abscissa of blood pressure; *u*, urine flow; *t*, time in one-second intervals. Exposure and faradic irritation of the left superior sympathetic ganglion.

lobe extract as shown on Schäfer and Herring's tracings, and such as we ourselves have often observed.

In some cases immediately after the exposure of the superior sympathetic ganglion there occurred some acceleration in the flow, to be explained, I presume, by the fact that some mechanical irritation of the ganglion is inevitable during the manipulations. The acceleration of flow subsequent to faradization of the ganglion begins, as after an intravenous injection of hypophyseal extracts, after a short interval of time. This interval in some observations was quite insignificant, only a few seconds long, but in others it lasted from two to four minutes. If it occurred immediately, the reaction was usually pronounced, indicating either greater activity of the gland in the particular animal or a more successful discharge. Occasionally the drops of urine have increased ten-fold in their rapidity and after a long interval have returned again to the normal or even below it.

Repeated stimulations in the same animal with the same strength of current resulted in a reaction which, as a rule, was not so obvious, or even, as in the case of experiments No. 15 and No. 20, was, altogether, absent; but if on these subsequent stimulations the current was increased, it evoked an acceleration in the flow of urine, in some cases even on the second or third excitation. Doubtless the gland may become exhausted and in some instances after a repeated stimulation had ceased to produce any further reaction, an acceleration of flow could be produced after an intravenous injection of pituitary extract.

With regard to the influence of the ganglionic stimulation on the animal's blood pressure, we have almost invariably observed a primary fall and subsequent rise after each of the primary stimulations. These haemodynamic responses behaved much as the diuretic ones, for a repeated stimulation with a current of equal intensity produced a less obvious response or none at all (e.g., experiment No. 15). However, applying a current of a greater intensity at each subsequent stimulation, the modifications of blood pressure produced were as determinate as those occasioned by the first excitation (e.g., No. 22 and No. 29).

It should be emphasized that the acceleration of urine flow is independent of the elevation of blood pressure, for, as a matter of fact, it often takes place at the moment of the lowest depressor phase (experiments No. 15 and No. 29). It might be objected that the increased flow was due not to an augmentation of the secretory discharge of the kidneys, but that we are dealing with a mechanical modification of the bladder capacity from some reflex or other action. However, in the

experiments with a high transection of the thoracic cord, there was a complete paralysis of the abdominal walls so that by no possibility could contractions of the abdominal musculature or other movements have influenced the urinary discharge.

In all these experiments the urine was tested for the presence of sugar before and after stimulating the ganglion. In a few cases sugar was found in small quantities before the stimulation, due to the glycosuria produced by and maintained after the spinal cord transection. Still, even in these cases, after stimulation of the sympathetic ganglion the quantity of sugar was much augmented. In these experiments, in which before the stimulation the urine was sugar free, sugar usually appeared after the stimulation, just as was observed in the experiments of Weed, Cushing and Jacobson.

The following are some of the typical protocols of the second series of experiments.

Experiment No. 15. Cat, weight 2700 grams.

Transection under ether of the spinal cord at 4th thoracic segment, 4 days previous to the beginning of the experiment. All aseptic precautions observed. No complications.

July 10, 1914. Division of both vago-sympathetic trunks under urethan anaesthesia. Catheter introduced into the bladder and cannula into the right carotid artery. Dissection of tissues in the region of the left superior sympathetic ganglion.

11.25 a.m. Kymographion record started. Urine flow in drops with intermissions varying from 18 to 25 to 30 seconds; blood pressure 98 mm.; a small trace of sugar in urine.

During the exposure of the ganglion and coincident with the period stimulating it, a few drops of urine issued with intermissions of 9 to 2 seconds.

11.30 a.m. Faradic stimulation of the ganglion for 20 seconds. This was almost immediately followed by an acceleration of urine flow, with drop intermissions reduced to only 2, 1, and even $\frac{1}{2}$ second each (fig. 2).

Blood pressure previously at 98 fell to 84 and then rose to 108 mm. The period of accelerated urinary flow coincided with the depressor phase.

11.50 a.m. Intermissions between the drops of urine now average 35 seconds. *Second stimulation* of the ganglion with the same strength of stimulus without appreciable effect on urine or blood pressure except for a slight depressor response.

12.17 p.m. *Third stimulation* with the same strength of current, but for a longer period. No appreciable effect except for a slight depressor response. Urine shows a heavy sugar reduction.

12.25 p.m. Intravenous injection of 1 cc. of Armour's "pituitary liquid."² No effect on urine; slight modification of blood pressure from 88 to 76 to 94 mm.

² This preparation, as we can conclude from a series of our observations, does not usually lead to the polyuria which is so characteristic of other hypophyseal preparations.

Experiment No. 20. Cat, weight 3700 grams.

Under ether; transection of the spinal cord at T. 4, six days previously. All aseptic precautions taken.

July 22, 1914. Urethan, 7.4 grams. Both vago-sympathetic trunks sectioned in the middle of the neck; a catheter inserted into the bladder and a cannula into the right carotid artery. Dissection of tissues in the region of the superior sympathetic ganglion.

10.30 a.m. Kymographion registration started. Drops of urine issuing with an average interval of 35 to 45 sec. Blood pressure 98 mm. No sugar in urine.

10.51 a.m. Exposure and stimulation of the ganglion by faradization. An almost immediate acceleration of urine flow with shortened interval to 11 or 12 seconds (fig. 3). Blood pressure rises without primary fall from 98 to 108 mm. Urine shows a marked reduction of sugar.

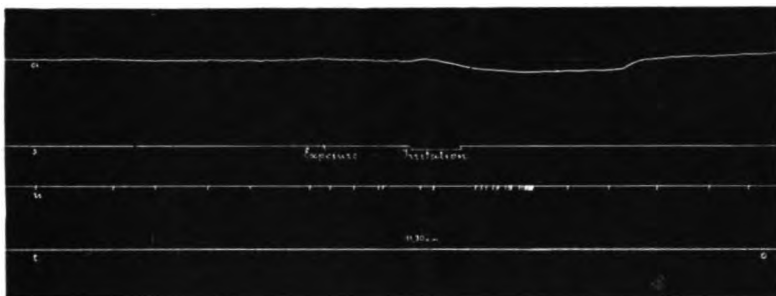


Fig. 2. Exp. N 15. *a*, arterial blood pressure; *s*, signal and abscissa of blood pressure; *u*, urine flow; *t*, time in one-second intervals.

11.35 a.m. The intermissions between drops average about 90 seconds. *Second stimulation* with an identical current. Cessation of urine flow. Slight pressor response from 96 to 108 mm.

12.24 p.m. No urine appearing. *Third stimulation* with the same strength of stimulus. No urine flows. Slight depressor response from 92 to 82 to 92 mm.

12.36 p.m. Intravenous injection of 0.9 cc. of "hypophysin." Three minutes later drops of urine began to issue with intermissions of 60 to 70 seconds. Modifications of blood pressure from 92 to 74 to 116 mm.

Experiment No. 25. Cat, weight 1700 grams.

Transection under ether of the spinal cord at T. 4, three days previously. All aseptic precautions taken.

August 2, 1914. Anaesthesia by interrupted intratracheal insufflations of ether. Both vago-sympathetic trunks sectioned; catheter and canula as before; dissection of the superior sympathetic ganglion.

10.45 a.m. Kymographion tracing started. During the 6 minutes previous to stimulating the ganglion, there was complete cessation of urine flow. Blood pressure at 90 mm.

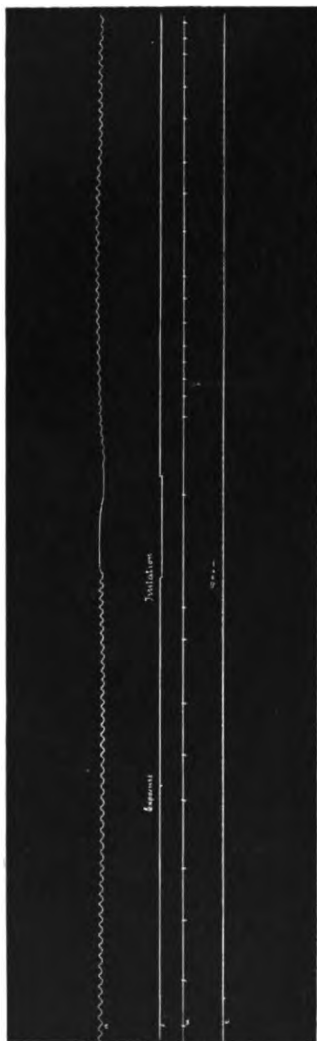


Fig. 3. Exp. N 20. *a*, arterial blood pressure; *s*, signal and abscissa of blood pressure; *u*, urine flow; *t*, time in one-second intervals.

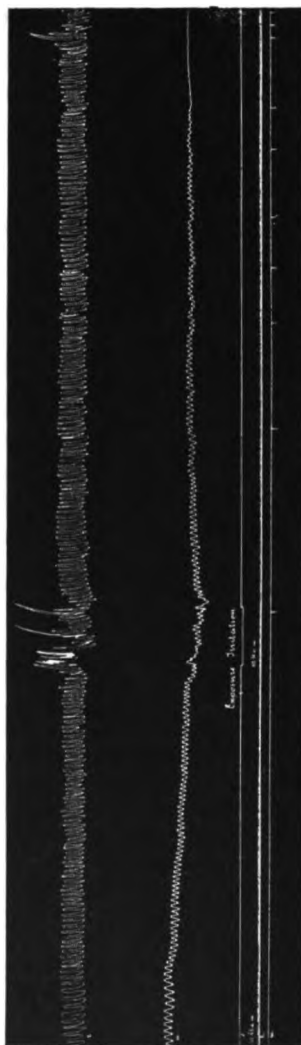


Fig. 4. Exp. N 25. First stimulation of respiration; *a*, arterial blood pressure; *s*, signal and abscissa of blood pressure; *t*, time in one-second intervals; *u*, urine flow. No urine was being secreted before the irritation.

10.51 a.m. Stimulation of the ganglion by faradization. At the last moment of the excitation corresponding with the period of lowest blood pressure, appeared the first drop of urine; the second drop came out two minutes later, the third in one minute, and then drops issued in 30 to 15 to 8 second intervals. The blood pressure showed merely a short depressor phase from 90 to 64 to 90 mm. (fig. 4).

11.38 a.m. The intermissions between drops now average about 50 to 85 seconds. *Second stimulation* of longer duration applied to the ganglion followed by a primary suppression of urine for 3 minutes succeeded by an acceleration with issuance of drops every 35 to 20 to 10 seconds. Blood pressure reaction from 84 to 66 to 80 mm.

12 noon. Average interval between drops equals 47 seconds.

Third stimulation by a stronger current. Urine flow checked for 90 seconds followed by an acceleration with intervals of 20 to 8 to 5 seconds. Modification of blood pressure from 80 to 62 to 86 mm. Heavy sugar reaction in urine towards the end of the experiment.

Experiment No. 29. Cat, weight 2000 grams.

Spinal cord transection at T. 4, three days previously. All aseptic precautions taken. No complications.

August 9, 1914. Intratracheal anaesthesia of ether without interruptions and usual preparation with division of both vago-sympathetic trunks.

3.04 p.m. Kymographion registration started. Drops of urine issuing every 37 to 47 seconds; blood pressure 116 mm.; urine negative for sugar.

3.18 p.m. *First stimulation* (coil at 1.5 cm.) followed by acceleration of urine, the intervals being reduced to 28 and 25 seconds. Blood pressure reaction from 116 to 82 to 144 mm.

3.34 p.m. Intermissions in urine drops lengthened to 30 to 42 seconds. *Second stimulation* with a stronger current (coil at 2 cm.) followed by increase of drops issuing at every 13 to 10 seconds. Characteristic blood pressure response from 114 to 48 to 140 mm. (fig. 5).

4.17 p.m. Drops of urine have slowed to every 40 to 50 seconds. *Third stimulation* with a stronger current (coil at 2.5 cm.) followed again by an acceleration of urine flow; drops issuing every 30 to 23 seconds. Typical blood pressure response from 104 to 80 to 134 mm. A large amount of sugar present in the urine.

Equally positive results were obtained in 8 of the 15 experiments in which a preliminary spinal transection was performed. In 5 of the remaining 7, there was a certain acceleration of the flow, though it was too insignificant to speak positively regarding it; whereas in the remaining two experiments there was no acceleration of the flow whatever.

These negative results are possibly no more numerous than might be expected, for Schäfer and Herring have pointed out that, in spite of all precautions, the "operative procedure and the effect of the anaesthetic combined, frequently produced suppression of urine flow, so that in some experiments this part of the record is negative throughout."

In our experiments, the operative traumatization was necessarily far

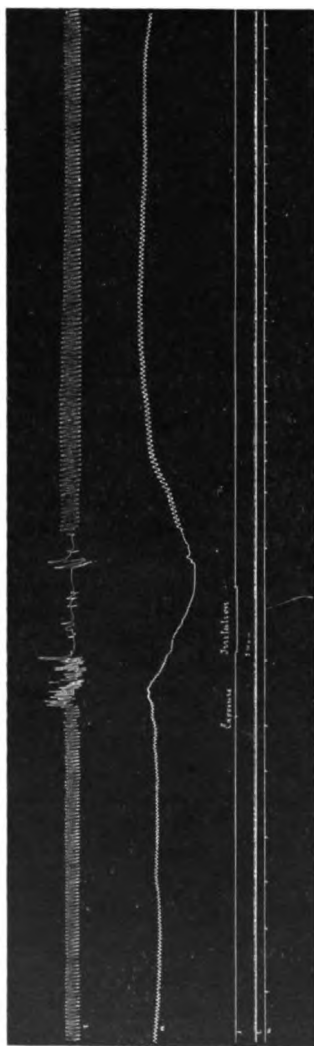


Fig. 5. Exp. N 29. *r*, respiration; *a*, arterial blood pressure; *s*, signal and abscissa of blood pressure; *u*, time in one-second intervals; *u*, urine flow. At 3.34 p.m. the second stimulation of the ganglion.

greater than that inflicted by Schäfer and Herring, whose total operative procedure consisted merely in the insertion of the cannula into the carotid and of a catheter into the bladder. Furthermore, in place of injecting intravenously a comparatively large quantity of hypophyseal extract of other animals, we attempted to produce a more or less natural secretory discharge of the pituitary gland of the experimental animal itself. Under these conditions we might expect a far less pronounced reaction, as well as considerable fluctuations of it, depending on the state of the organ in each separate animal at the time of the experiment. In all likelihood, the secretory power of the gland is influenced by the state of the organism, and the preliminary spinal cord transection may have considerably reduced our chances of producing a normal discharge from the pituitary gland. Thus the unfavorable factors, to which Schäfer and Herring refer, must have been considerably increased by the major procedure necessary for our experiments.

In addition to the above-mentioned factors, there is the further consideration pointed out by Schäfer and Herring, namely, the presence of a substance in hypophyseal extracts which acts as a vasoconstrictor and another as a vasodilatator; consequently in some cases "the vasoconstrictor may overpower the vasodilatator substance in acting upon the renal vessels, and this would naturally lead to a diminished secretion of urine as long as the constriction of renal vessels lasts, and for that period, at least, the extract would be antidiuretic rather than diuretic." Very likely the discharge of different substances of the pituitary gland is occasioned by strictly determinate different factors in the organism, and possibly they are modified by variable nervous impulses passing to the gland. A hint as to this can perhaps be found in the fact that in some of our experiments, after a repeated irritation, instead of an acceleration of urine flow a temporary retardation occurred. We of course cannot exclude the possibility of stimulation of different parts of the gland through irritation of one or another part of the cervical ganglion. From this point of view the failure to obtain diuresis in a number of our experiments can possibly be explained.

As a matter of fact, the negative records in the series of experiments do not serve to offset the positive responses. But can even the positive records be looked upon as definite? And can the diuresis seen by us be really explained as the effect of hypophyseal secretion, discharged by the stimulation of the superior sympathetic ganglion? This deserves an affirmative answer, inasmuch as our experiments have involved a

total exclusion of all nervous impulses from the stimulated ganglion down to the kidneys and hence we can conceive of only one way of influencing their activity and that is through the blood by the diuretic hormone of the posterior lobe. The gland that produces the substance, in spite of the division of both vago-sympathetic nerves and the transection of the spinal cord at the fourth thoracic segment, remains connected by a nervous pathway with the superior sympathetic cervical ganglion. As to its being actually the pituitary body is demonstrated by all reactions typical for its secretion, for after the stimulation of the sympathetic cervical ganglion (1) there usually occurs a characteristic haemodynamic response; (2) as was observed by Weed, Cushing and Jacobson, as well as by myself, glycosuria is provoked; and finally, (3) a polyuria, resembling that produced by intravenous injection of pituitary body extracts, frequently occurs.³

Thus, the positive records of my experiments fully coincide with the data of my predecessors in this laboratory but indicate further that stimulation of the superior cervical sympathetic ganglion leads to a discharge of hypophysial secretion which produces diuresis, as well as glycosuria.

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³ Lately Camus and Roussy (1) have published some experiments to prove that the "polyurie dite hypophysaire" is due to a lesion "de la région interpedonculaire de la base du cerveau" rather than to that of the pituitary body itself. Similarly Aschner and others have chosen to explain the so-called hypophyseal glycosurias, adiposity, drowsiness and so on to injury of predicated centres in the floor of the third ventricle. The main results of the experiments of Camus and Roussy are that polyuria is produced not only in cases of experimental lesion of the pituitary body itself, but also in lesions of the basis cerebri situated immediately behind it, and that in the latter case the lesion was accompanied by polyuria even after a preliminary extirpation of the pituitary gland. On the contrary, in cases where the extirpation of the hypophysis was performed with special precautions, so as not to traumatize the cerebral base, it produced in animals no polyuria whatever. These observations deserve consideration and repetition, but the prevalent view of most investigators is that these phenomena are of hypophyseal rather than of encephalic origin.

THE ORIGIN OF THE CARDIAC IMPULSE IN THE TURTLE'S HEART

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The cardiac physiology of the cold-blooded heart has been found applicable to the mammalian heart in practically all of its details. This fact has lead certain investigators to doubt the propriety of assigning the seat of impulse formation to the sino-auricular node in the mammalian heart unless it could be shown that the homologous structure of the cold-blooded forms was also a motor center. Erlanger (1) in particular has voiced this view.

Since the work of McWilliam, Gaskell and Engelmann it has been accepted with but few exceptions (2) that the heart beat in the cold-blooded animals arises in the sinus. This we have ourselves confirmed by the newer electrical methods (3). So far as we are aware, however, no attempt has ever been made to discover whether the sinus acted as a unit or whether certain of its parts were preeminent in automaticity. Certainly this point was not touched in our previous publication.

With the bulk of the recent work emphasizing the function of the sino-auricular node as pacemaker in the mammalian heart, two important questions from the comparative physiological standpoint arise: first, what part of the primitive heart does the sino-auricular node represent; and second, does the beat originate in this homologous structure in the lower vertebrate forms.

As to the first of these questions there are now anatomical investigations extant which we believe furnish a correct answer. His (4), as is well known, found by embryological methods that the portion of the mammalian heart designated by him as sinus reuniens represented the sinus of the lower forms. Although he found a complicated fusion of sinus and auricle he nevertheless felt able to locate the boundaries of the former. The sulcus terminalis on the exterior and the taenia terminalis on the interior marked the line of separation between the sinus reuniens and the pectinate musculature of the auricle.

The sino-auricular node, being found in the sulcus terminalis, would then, according to the conceptions of His be a structure occurring at the sino-auricular junction. But even with this knowledge one could not be quite sure whether the node really belonged to the sinus side of the junction. It might of course be an auricular structure shifted to this position during the period of cardiac development. The exact location of the sino-auricular node in the mammalian heart has been determined in a very important piece of work done by Oppenheimer and O, penheimer (5). These investigators examined with great care the hearts of two fetuses and one child. In these hearts they found

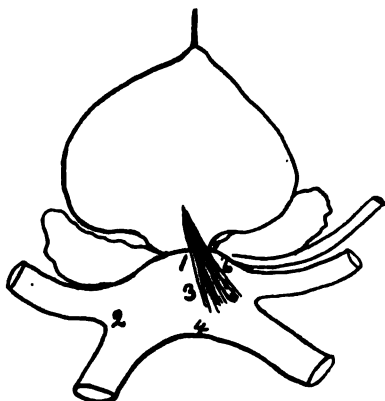


Fig. 1. Ventral outline of turtle's heart with the ventricle pulled up and forward to expose the sinus. Figures indicate the position of the electrodes on the sinus.

the venous valves, structures which in lower forms separate the sinus from the auricular canal. That Oppenheimer and Oppenheimer actually found the venous valves can scarcely be doubted since they were identified by shape, structure and location. The identification of the valves of course gave a landmark for the exact location of the sino-auricular node. The node was easily discernible at the base of the venous valves in the region covered with a thick endocardium; in other words in the tissue which corresponds to the sinus venosus of cold blooded vertebrates.

The exact location of the sino-auricular node seems then to be on the sinus side of the sino-auricular junction. This region of the lower forms has been examined histologically by Keith and Flack (6) and by Keith and Mackenzie (7). They report at the sino-auricular (sino-canalar) junction, a network of fine, primitive palely staining fibers evidently the homologue of the sino-auricular node in the mammalian heart. In the tortoise Keith and Mackenzie describe this nodal tissue as being in intimate connection with the right auricle although its chief center is at the termination of the pulmonary vein. This vestibule of the pulmonary vein they have shown to be of sinus origin. The nodal tissue really makes a circle around the sino-auricular junction with its chief mass on the left side of this ring.

The second question, that in regard to what part of the cold-blooded sinus functions as pace maker, we have taken up in the present paper. Our method has been to determine the point of initial negativity in the sinus by means of the string galvanometer. Large mud turtles, *Chelydra serpentina*, were used. The animals weighed about 30 pounds and the sinus was often found to be as much as 4 cm. wide. We have now studied eight of these hearts and the results have been consistent enough to justify reporting the series.

After pithing, the carapace was removed. The heart was exposed and the ventricle raised and tilted forward thus completely exposing the sinus region. Figure 1 gives an outline of the parts with numbers showing the position of the electrodes. These were non-polarizable of the zinc-zinc sulphate type. From the end of the glass tubes forming the electrodes, hung woolen threads soaked in salt solution which could be applied to any part of the heart. They were watched throughout the experiment to

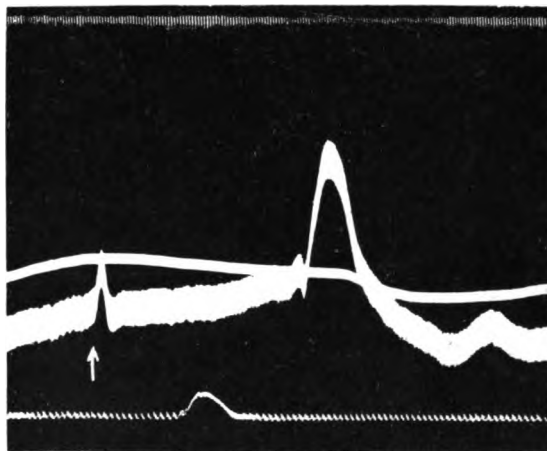


Fig. 2. A comparison of the sino-auricular ring (1) with the right edge of the sinus (2). The right hand electrode is on point 1, hence the up stroke of the sinus wave indicates primary negativity of the sino-auricular ring. The ventricle has been tied off and is quiescent. The second wave is auricular. An arrow marks the sinus wave. The other lines are the signals, the upper from the auricle, the lower from the observer.

insure their remaining in the same position. The contraction of the sinus was identified either by referring to the auricular contraction which was recorded by air transmission in front of the photographic slit, or by means of a similarly recorded signal from the observer. In all cases after records were taken from the undisturbed heart the second Stannius ligature was laid and the observations repeated. This series has been considered the most important since in some cases the T wave of the ventricle had obscured the sinus deflection. A sen-

sitive string was used, 1 millivolt deflecting the string through 29 cm. at the distance of the photographic slit.

The positions at which the electrodes were placed may be seen by referring to figure 1. Point 1 was over the left ventral quarter of the sino-auricular ring; 2 was at the extreme right of the ventral wall of the sinus; 3 was slightly to the left of the middle of the ventral sinus wall; 4 was at the posterior edge of the ventral sinus; and 6 was over the extreme left edge of the sino-auricular ring. Our results are briefly as follows:

Point 1 was negative before point 2 in 8 of 8 experiments.

Point 1 was negative before point 4 in 7 of 8 experiments.

Point 1 was negative before point 6 in 6 of 7 experiments.

Point 1 was negative before point 3 in 4 of 5 experiments.

Point 1 was thus found to precede regularly all other parts of the sinus. In other words it would seem that in the eight turtles examined by us the cardiac impulse was in all

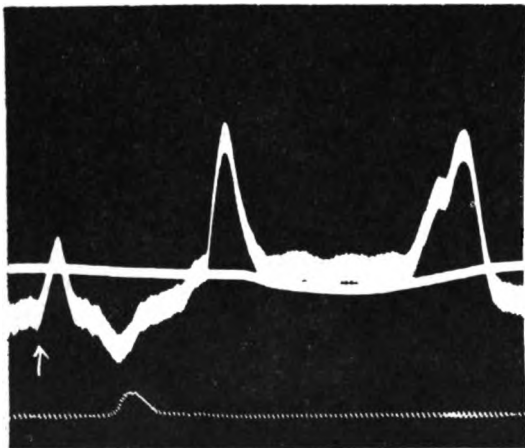


Fig. 3. Sino-auricular ring (1) compared with the posterior edge of the sinus (4). Up stroke of the sinus wave indicates primary activity of the sino-auricular ring. The sinus wave is here followed by the auricular and ventricular deflection.

cases being initiated in some part of the sino-auricular ring. It will be noted that point 1 was preceded by points 6, 4 and 3 in one case each. These exceptions all arose in one experiment in which it was found that the beat was arising at the extreme left edge of the sino-auricular ring under electrode 6. As the excitation spread points 3 and 4 became involved before 1, but as might be expected 1 still preceded 2. That the region under electrode 6 should at times be pacemaker is not surprising since it was here in the tortoise that Keith and MacKenzie (7) found the largest masses of specialized tissue. They noted that there was a tendency even in the cold-blooded forms for this tissue to shift toward the right and thus come in still more intimate rela-

tion to the right auricle. Such a shifting might well explain the fact that point 1 usually precedes point 6 in activity in the forms studied. Even if the pacemaker is found at times in the region of point 6 it in no way modifies our conclusion that the beat starts in the sino-auricular ring presumably in the left portion. Point 3 was compared with points 2 and 4 and found regularly to precede them in activity. These leads served as a control and further proof that excitation was spreading from the sino-auricular junction.

Our results justify the conclusion that even in the cold-blooded hearts the cardiac impulse originates in a definitely localized portion. This motor center is in the sino-auricular ring where Keith and Mackenzie have described a specialized muscle tissue resembling in most of its characteristics that found in the nodes of the mammalian heart. All the data at present available, both anatomical and physiological support the generalization that in all vertebrate hearts the seat of cardiac impulse formation is located at the sino-auricular junction in masses of the so-called specialized tissue.

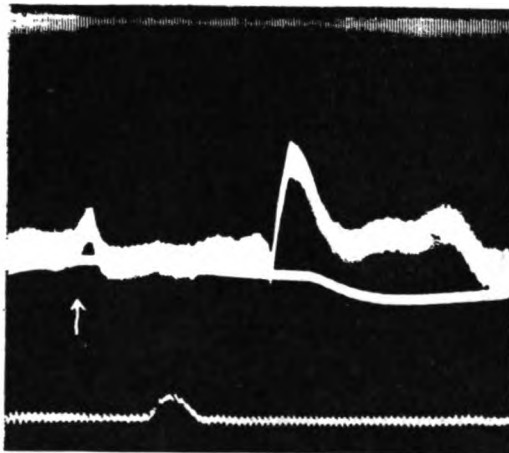


Fig. 4. A comparison of the ventral part of the sino-auricular ring with its extreme left margin. Up stroke indicates primary negativity of the ventral portion rather than the left edge of the ring. The ventricle has been tied and its deflection does not appear.

SUMMARY

Electrocardiographic examination of the turtle's heart shows that the initial point of negativity during each cardiac cycle is in the sino-auricular junction. It is in this region that specialized tissue is found, both in the cold-blooded and mammalian hearts. All vertebrate forms then seem to agree in having the pacemaker of the heart located at the sino-auricular junction in masses or nodes of specialized tissue.

Since writing the above our attention has been called to an important paper by Laurens on the "Connecting Systems of the Reptile Heart" (*Anatomical Record*, 1915, ix, p. 427). Laurens finds that in the tortoise heart the best connection between the sino-auricular ring and the right auricle is at the position of the right venous valve. On the left side the fibers are divided between auricle and septum. Our experiments, which to our surprise showed the pacemaker to be somewhere to the right of the left venous valve, are therefore in harmony with Lauren's anatomical findings. It is probable that if different parts of the sino-auricular ring were compared the pacemaker would be found near the right venous valve. Our work of course has not taken up this point but has been content with showing that the seat of impulse formation was in the sino-auricular ring rather than in the body of the sinus.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE
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NO. 267

IONIC ANTAGONISM IN SENSORY STIMULATION

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This note is concerned with a demonstration of the fact that laws of salt antagonism are applicable to certain phenomena of sensory stimulation by electrolytes. Such an antagonism may readily be demonstrated for the salts of sea water when marine animals (Holothurians, Ptychodera) are the subject of experiment. According to Parker and Metcalf (1) the reactions of the earthworm *Eisenia foetida* to mixtures of NaCl and CaCl_2 are not such as to indicate an antagonistic effect of these salts in their action on the chemoreceptors of the worm; but their study dealt with only a few concentrations. The data with reference to human taste are in too chaotic a state and too complex to admit of analysis at present.

Material used in the present experiments comprised some 54 frogs, *Rana pipiens*, ranging in length (snout to anus) from 60 to 87 mm. The experiments were all made upon autumn frogs, during October and November, 1914. The method followed was, to all intents and purposes, that of Cole (2). The frogs were prepared by pithing and hanging upon a platinum hook passed through the lower jaw; care was taken that the frogs were in good condition and that the feet were not abraided. About one-half hour elapsed between pithing and the first stimulation trials, during which the skin was kept moist with tap water.

In measuring the reaction time a frog's foot was immersed in a given solution up to the proximal end of the digits. It is worth while mentioning that the area of the foot exposed to the solution does not have to be constant, since only very slight and inconstant variations in reaction time are found when the responses are compared for immersion of (a) the whole foot and (b) one-half the length of the digits. The salt studied were NaCl, KCl, and CaCl_2 . The trials on individual frogs were so rotated among solutions of these salts that fifteen reaction times

TABLE I

Reaction time of frog's foot, seconds. R. and L. signify right and left feet respectively

October 23, 1914 Frog 47 (62 mm.).
(19°6)

October 24, 1914. Frog 49 (66 mm.).
(20°0)

SOLUTION	R	L	SOLUTION	R	L
NaCl, 2M	11.9	6.2	CaCl ₂ , 0.1M	∞ *	∞
	6.2	10.5		∞	∞
	7.1				∞
CaCl ₂ , 1M	8.0	6.7	NaCl, 1.5M	4.3	8.3
	12.9	11.4		6.3	12.0
		10.6		13.0	
NaCl, 2M, 50 cc. {	10.1	11.2	NaCl, 0.75M	58.9	45.0
CaCl ₂ , 1M, 50 cc. {	14.4	16.0		34.5	55.1
	14.8				28.7
NaCl, 0.5M	44.2	65.6	NaCl, 2M, 25 cc. }	10.3	10.6
	42.5	29.4	CaCl ₂ , 1M, 75 cc. }	13.9	15.2
		25.7		9.7	

* N.B. ∞ = no reaction (i.e., infinite reaction time).

TABLE II

Reaction time of frog's foot, seconds. Averages of 15 measurements

SALT	NaCl	KCl	CaCl ₂
Conc.			
2.0M	11.5		
1.5M	8.31		
1.0	26.8	4.36	10.4
0.75	44.4		14.3
0.50	40.8	5.36	37.3
0.25	∞	10.5	149.
0.10		25.6	∞

TABLE III

Reaction time to mixtures of NaCl and CaCl₂.

NaCl, 2M and CaCl₂, 1M.

xcc.A + ycc.B

90A + 10B	75A + 25B	50A + 50B	25A + 75B	10A + 90B
13.4	14.3	13.3	11.8	11.3

were measured for each concentration, using three frogs to give five reactions each, but the order in which the successive salts were tried was varied from frog to frog in such a way as to rule out any after effect of a particular solution. By way of illustration the records of frogs 47 and 49 are given in detail (Table I). The temperature during these experiments varied from 18° to 21°. After each test the foot concerned was washed in tap water.

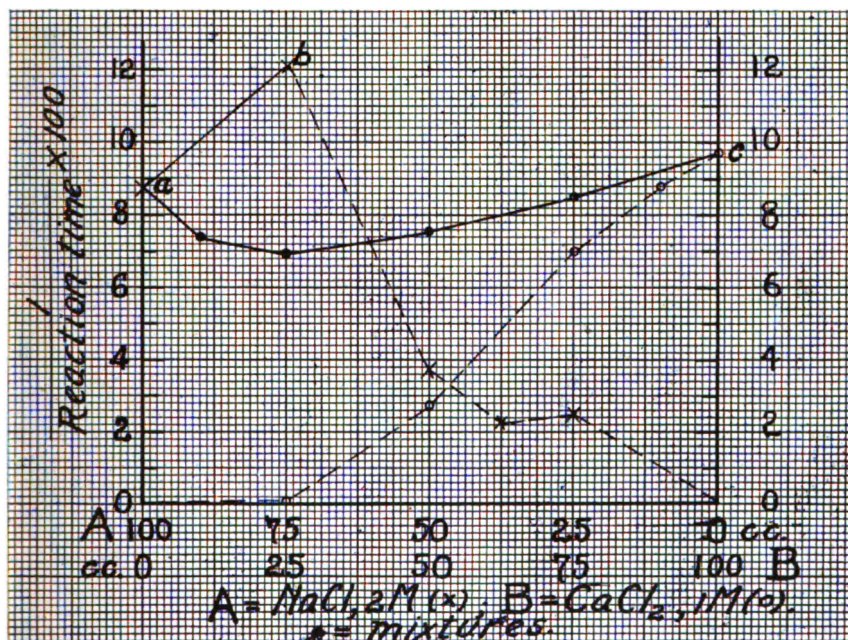


Fig. 1. Reaction time of the frog's foot to mixtures of NaCl and CaCl₂. For explanation, see text.

Data for solutions of the single salts, NaCl, KCl, and CaCl₂, are summarized in Table II.

If the results of Cole(2) in a similar study be compared with the values of Table II, it will be noted that his frogs were rather less reactive and more variable than mine, and further that there is not a very good concordance in the individual averages. This is mainly to be accounted for, I believe, by the fact that Cole's work dealt with spring frogs; size variations are also liable to be important.

It was found at the beginning of these experiments that, while 0.5M NaCl gave a weak but measurable stimulus, sea water (0.6M) was totally inefficient in this respect. Mixtures of NaCl and CaCl_2 were then studied, with the results given in Table III, and shown graphically in figure 1. In figures 1 and 2 the *reciprocals* of the reaction times have been plotted; the method of representation is a variation of that suggested by Osterhout (3), with the addition of the dilution curves for the individual components of the mixtures; the variation consists in

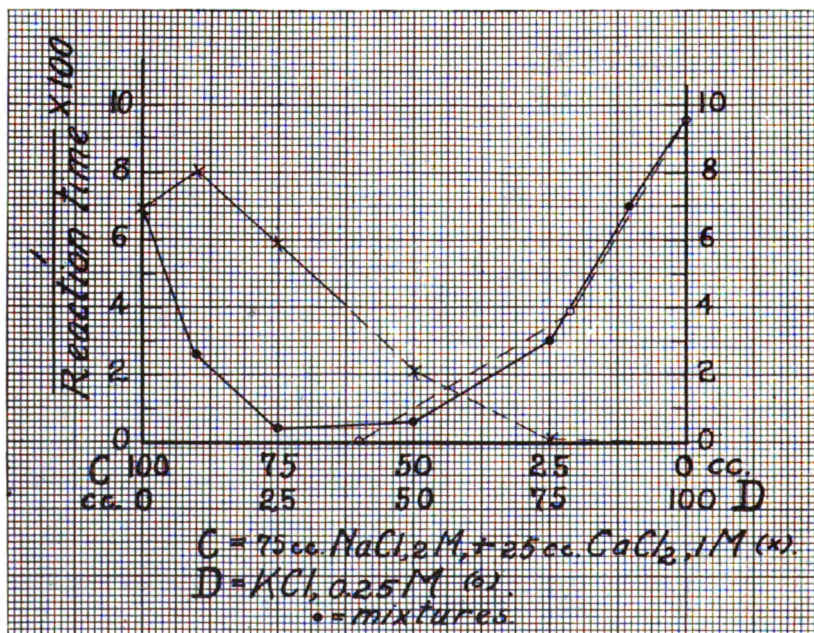


Fig. 2. (See text.)

plotting, not molecular percentages, but volume percentage compositions, as abscissas. Since reciprocals of reaction times (i.e., "stimulating powers" of the solutions) are plotted, antagonism of one component of the mixture by another would be shown by the curve for the mixtures lying below the theoretical "curve of no mutual action," and by its being convex toward the axis of abscissas; but the occurrence of a convexity in this sense does not necessarily indicate an antagonistic effect, because the nature of the base line for measuring antagonism in Osterhout's method cannot in general be determined with accuracy.

The "base line" referred to is given by Osterhout (3), for the case when $A C$ and $B D$ are equal, as the straight line connecting the ends of the ordinates which represent the activity of the solutions of each of the components of the mixture. As a matter of fact, such a base line, giving the additive effect of mixtures between solutions A and B , were there no mutual interaction of the substances, could only result if the *dilution vs. effect* curves for the individual components were of exactly identical forms;¹ a base line of the required significance might then be calculated. This point is equally applicable when the percentage composition of the mixtures is expressed in terms of the molecules present.

TABLE IV
*Reaction time to dilutions of NaCl + CaCl₂,
75 cc. NaCl, 2M + 25 cc. CaCl₂, 1M and Water.*

x cc. C			+ y cc. W	
90C + 10W	75C + 25W	50C + 50W	25C + 75W	10C + 90W
12.5	16.9	49.7	99.5	∞

TABLE V
*Reaction time to mixtures of NaCl, CaCl₂, and KCl.
75 cc. NaCl, 2M + 25 cc. CaCl₂, 1M and KCl, 0.25 M.*

x cc. C			+ y cc. D	
90C + 10D	75C + 25D	50C + 50D	25C + 75D	10C + 90D
38.4	250	166	33.5	14.3

In the case recorded here the activity of the mixed solutions was higher than that of the corresponding concentrations of the single substances, except for the mixtures having 75 per cent and 90 per cent NaCl; the curve has a minimum at 75 cc. NaCl + 25 cc. CaCl₂. It is important to note that this effect of the CaCl₂ is apparent in a mixture of which the CaCl₂ component has *by itself* no stimulating action—hence the "curve of additive effect" would for this case be that portion (a, b) of the NaCl curve which is drawn as a full lines (cf. also figure 2).

¹ It is hardly necessary to remark that such a condition is in reality never found with solutions of different salts.

This mixture of NaCl and CaCl₂ was used as one component of mixtures containing KCl. The dilution curves for these components and for the three-salt combinations are shown in figure 2, plotted from the data of Tables IV and V. In the mixture containing 75 cc. (NaCl + CaCl₂) and 25 cc. KCl there is apparent a minimum of stimulation; the solution contains the three salts in the ratio Na: K: Ca :: 12: 1: 8. The Na: K ratio (12: 1) at this point is not far from that found by Loeb (4) for antagonism in various concentrations of Na and K salts (17: 1). This mixture stimulated to a measurable extent, while sea water did not; it is uncertain whether the lower total concentration² of the sea water or the presence of additional salts determined its nonstimulation.

From the fact that antagonism is evident in the stimulation of the frog's foot by salts, it seems legitimate to conclude that in normal stimulation the essential step includes the penetration of the surface layer of the receptor by the stimulant.

POSTSCRIPT

I have recently (December 4, 1915) received a copy of a paper by Professor Osterhout in which an attempt is made to correct the determination of the "curve of additive effect." In this paper, (*Bot. Gaz.*, vol. 60, 1915, pp. 228-234) the method of measuring antagonism originally proposed as generally valid, is apparently restricted to *toxic* effects. It seems only fair to state that the present paper was written without knowledge of Professor Osterhout's later communication. As a matter of fact, the existence of the complication above referred to, and the necessity for detailed consideration of the dilution curves, were first pointed out by myself in conversation with Professor Osterhout in October, 1914.

Agar's Island, Bermuda,
December 4, 1915

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² There is no a priori justification for any expectation that the salt ratios at a point of maximum antagonism will be identical for a range of (total) concentrations.

CONTRIBUTION TO THE PHYSIOLOGY OF THE STOMACH

XXXI. THE EFFECT OF EXPERIMENTAL PARTIAL STENOSIS OF THE PYLORUS ON THE MOTILITY OF THE EMPTY STOMACH

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It is well known that partial stenosis of the pylorus in man leads to altered motility, tonicity (and secretion) of the filled stomach to a greater or less degree. Some attempts have been made to reproduce this condition in experimental animals in order to analyze their physiological significance. Pernice (1) in 1890 produced a partial stenosis of the pylorus in dogs by tying a sterile string under some tension around that sphincter. He merely noted the constitutional effects of his procedure on the animals. Roger and Garnier (2) in 1906 completely occluded the pylorus of three dogs by means of a rubber band. The dogs died before the fifth day and presented stomachs greatly distended with gas and serous exudate. Tuffier and Bonamy (3) in 1898, also working on dogs, endeavored to produce varying degrees of stenosis using silk ligatures passed around the pylorus. Complete occlusion caused the death of one animal in seven days, which at autopsy showed merely a slightly dilated stomach. Partial occlusion produced practically no effects, the stomach evidently overcoming the hindrance. With partial chronic stenosis, it was found after twenty days, that the stomach filled the abdomen, "driving back all the viscera." The contents were liquid and of increased acidity. These authors venture the opinion that other results than these mentioned due to partial stenosis cannot be produced to any extent in less than two months duration. More recently Bolton (4) constricted the pylorus in cats, the results being varying degrees of motor insufficiency of the stomach, i.e., delay in emptying its contents into the duodenum. Hamburger and Friedman (5, 6) in their work on the genesis of gastric ulcer in dogs also constricted the pylorus by drawing a ligature about it. Depending on the degree of constriction they noted (a) little or no change in stom-

ach motility, acidity or size; (b) motor insufficiency, hyperacidity and hypertrophy and dilatation of the stomach, and (c) with complete occlusion death of the animal in from 48 to 120 hours.

Sick (7) made actual tracings (balloon method) of the contractions of the human stomach in a case of partial stenosis of the pylorus. Leonard (8), Case (9), Cole (10) and others have made extensive use of the X-ray in studying the motility of the filled stomach both under normal and pathological conditions. Hertz (11), Russell (12) and Maylard (13, 14) mention hypermotility, distension, etc., of the stomach in case of pyloric obstruction. Backman (15) presents an instance of pyloric stenosis with very large dilatation of the stomach but without motor insufficiency. Jonas (16) states that antiperistalsis of the stomach was almost always associated with pyloric stenosis.

These observations refer to the digestive motility of the stomach, that is, to the filled or partially filled stomach. Are the changes in gastric motility and tonicity in evidence only when food is present in the stomach and a part therefore of the normal mechanism by which the stomach tends to empty itself, or is there a fundamental change in the mechanism, i.e., chemical, sensory, neuromuscular, which persists even when the stomach is empty? In other words, does partial pyloric stenosis bring about changes in the motility and tonicity of the stomach which are present whether the latter is empty or not? The following experiments were carried on at the suggestion of Professor Carlson, with the view of answering this question.

EXPERIMENTAL PROCEDURE

Two young healthy female dogs, weighing between 5 and 8 kgm. were chosen. Both animals proved very tractable to the experimental technique and readily accustomed themselves to it. This consisted in swallowing a rubber condom attached to a firm rubber tube, which in turn was connected to a chloroform manometer, the latter transmitting the stomach contractions to a writing point on a slowly revolving drum. Tracings per os are to be preferred in this work to those taken per gastric fistula for the obvious reason that in the latter case we should have to deal with adhesions which might modify the stomach motility. The dogs, while the records were being taken, would lie quietly on their side, without any restraint of any sort, frequently sleeping complacently for periods of several hours—the work being carried on in a room as free from disturbing factors as possible. A series of normal tracings

were first recorded from each dog, comprising those taken immediately after feeding, and after twenty-four hour and forty-eight hour periods of starvation, whereupon the pylorus was partially stenosed according to a technique suggested by Dr. Carlson. The abdomen was opened

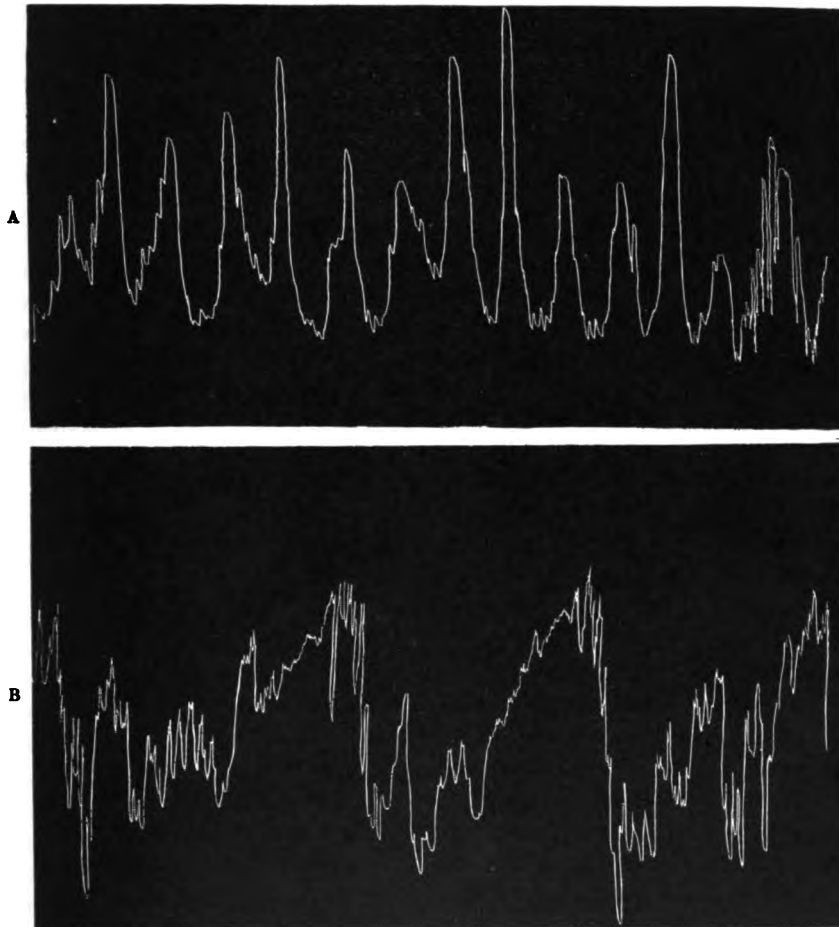


FIG. 1. Tracings showing contraction of the stomach of Dog A. Dog normal, the tracing, taken forty-eight hours after feeding, represents the height of a period of hunger contractions. B, same dog as in tracing A, forty-two hours after feeding, 30 days after production of partial stenosis of the pylorus, showing typical hypertonus and tetany periods of the empty stomach. Chloroform, Manometer. Time: 15 minutes.

under general anesthesia and the pyloric end of the stomach located. On being drawn gently through the incision, an area of serosa extending across the pyloric sphincter about $\frac{3}{4}$ cm. by $1\frac{1}{2}$ cm. was scarified, care being taken to avoid injury to the larger blood vessels of that region. Two rows of stitches running parallel to the long axis of the bowel were made, the second folding in the first, thus bringing the two scarified surfaces together and at the same time partially occluding the pyloric lumen. The method has these advantages, in that some pyloric obstruction is sure to be produced which will be firmly maintained by adhesions forming between the two raw surfaces. Furthermore, complete occlusion and loss of the animal is avoided, a termination frequently attendant upon placing ligatures around the pylorus. After the animal had recovered sufficiently from the effects of the operation—a matter of several days—another series of records were made duplicating the normal ones both as to conditions and periods of time. On one dog which we may designate as dog A, a second operation similar to the first was performed and a second series of tracings recorded. At all times the dogs were fed standard meals consisting of 170 grams and 180 grams respectively, of boiled, ground steak.

Dog A remained very well and active and was killed 35 days after the first operation and 14 days after the second. At autopsy the pylorus presented a lumen constricted by a hard tough mass of granulation tissue, the seat of the scarification and stitching. The stomach was not distended, but did show some hypertrophy. The gastric mucosa too lay in deep folds. There were no stomach contents, the animal having been given $\frac{1}{20}$ gr. of apomorphine previous to killing for another purpose.

Dog B became somewhat emaciated after stenosis, evinced a hypersensitivity of the gastric mucosa by frequently vomiting up the balloon while records were being made, and was found dead 7 days after the operation. Death could not have been due to complete pyloric obstruction as the animal continued to pass faeces after the operation. At autopsy the pylorus was constricted so that the stomach contents could be forced through it only with difficulty. The stomach itself was considerably dilated and contained much serous exudate, and meat from a previous meal.

Both dogs gave evidence of delayed emptying of the stomach after stenosis in that food remnants were frequently found in the stomach more than 24 hours after feeding, which were removed by a stomach pump before recording the tracing.

RESULTS

The normal contractions of the empty stomach were of the type described by Dr. Carlson, the predominating type depending on the length of starvation. As the method used offered no means of differentiating between pyloric and fundic contractions, the results obtained after partial stenosis represent movements of the empty stomach as a whole. Briefly stated they show hypertonicity and hypermotility. The degree of acidity and secretion was not determined in either dog. It might be proper, moreover, to designate here our conception of the use of these terms. By hypertonicity is meant (a) prolonged periods of tetanus beyond the normal—a feature which Carlson and Ginsburg have noted in infants with partial pyloric stenosis and pyloric spasms—and (b) an increase in the strength of the tonic contraction. By hypermotility an increase in the amplitude of the various hunger contractions. Whether marked hypertrophy of the stomach, atony and extreme dilatation would have resulted in these two dogs had the experiments been of longer duration cannot be answered and are points which should be solved.

It is thus clear that partial stenosis of the pylorus induces a hypermotility in the stomach irrespective of the presence of food in the stomach cavity. The hypermotility of the stomach during gastric digestion may be a temporary condition induced by the presence of the food and retardation of its passage through the pylorus, a condition similar to that of the small intestine above a region of obstruction. The fact that the hypermotility is present even in the empty stomach seems to show that the motor changes following mere mechanical obstruction of the pylorus are more fundamental and permanent.

The mechanism of this increased motility can as yet be only conjectured. Bacterial toxins from local foci of infection are excluded in these experiments, as the partial stenosis was produced aseptically in animals with normal stomachs and without subsequent infection. Dr. Carlson suggests nervous reflexes from the pylorus involving the entire stomach. Since the contractions of the empty stomach are primarily automatic, and in no case induced by the stimulation of the nerves in the gastric mucosa, it would seem that if the hypermotility obtained in these experiments is due to any chemical substance arising in the stomach its action on the Auerbach's plexus is probably via the blood.

An experimental animal or a patient, then, retains food longer in

his stomach than normal, if the pyloric sphincter be sufficiently occluded. With this he may suffer from hypermotility, hypertonicity, etc. And since our work shows that these conditions are present even when the stomach is empty, such an individual should, a priori experience more persistent and vigorous hunger pangs, if, as it has been shown, the sensation of hunger runs parallel to the vigor of stomach motility in the normal person at least. As this is apparently not the case, there is evidently a change in the sensory nervous mechanism of the stomach parallel with the motor and secretory changes.

It is interesting to note that tetanic contractions of the empty stomach obtained from dogs with partial pyloric stenosis closely resemble those taken by Luckhardt (18) on dogs during the severe hunger of experimental pancreatic diabetes.

CONCLUSIONS

1. Partial pyloric stenosis in dogs produces hypertonicity, and hypermotility of the empty stomach, even if, of but a few days or weeks duration. These motor phenomena closely simulate those seen in man (filled stomach) with pathological lesions of the pylorus.

2. The same conditions which lead to hypermotility, etc., during digestion, lead at the same time to increased motility of the empty stomach. In other word, partial pyloric stenosis appears to produce a neuro-muscular hyperactivity, independent of the presence of food in the stomach.

My deepest thanks are due Dr. Carlson for his keen interest and timely suggestions during the progress of this work.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH
XXX. THE TONUS AND CONTRACTIONS OF THE EMPTY STOMACH OF
INFANTS WITH CONGENITAL PYLORIC STENOSIS, PYLOROSPASM
AND CHRONIC VOMITING (MERYCISM).

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Pylorus spasm has been ascribed to a great variety of causes, including primary neurosis of the local motor mechanisms. The hyperperistalsis of the filled stomach, usually associated with spasms of the pylorus, may be a temporary condition due to the presence of food in the stomach. On the other hand, if pylorus spasm is simply an expression of primary hypermotility of the entire stomach, this condition of hypertonus and hypermotility should also be in evidence when the stomach is empty. A study of the motor conditions of the empty stomach may thus aid in determining some of the factors involved in hypercontractility of the pyloric sphincter.

In infants "rumination" is probably always secondary to chronic vomiting, which in turn may or may not be associated with pylorus spasm. If the vomiting is due to gastric hypertonicity and hypermotility, these conditions should also be present in the empty stomach, with or without the involvement of the pylorus.

Through the courtesy of Dr. C. G. Grulee we have been able to study the motor conditions of the empty stomach in two infants, one with congenital pyloric stenosis, and one with pylorus spasm, chronic vomiting and rumination. The results on these two cases are reported, not because we feel justified in concluding that the conditions found are typical, but rather with the hope that the work may be extended by others wherever such cases are at hand.

Case 1. Infant 3 months old, chronic vomiting and gradual loss of weight. Congenital pyloric stenosis. Gastro-enterostomy was made. The pylorus was found strongly contracted, and somewhat edematous and anemic. Before the operation record of the tonus and contractions of the empty stomach was made

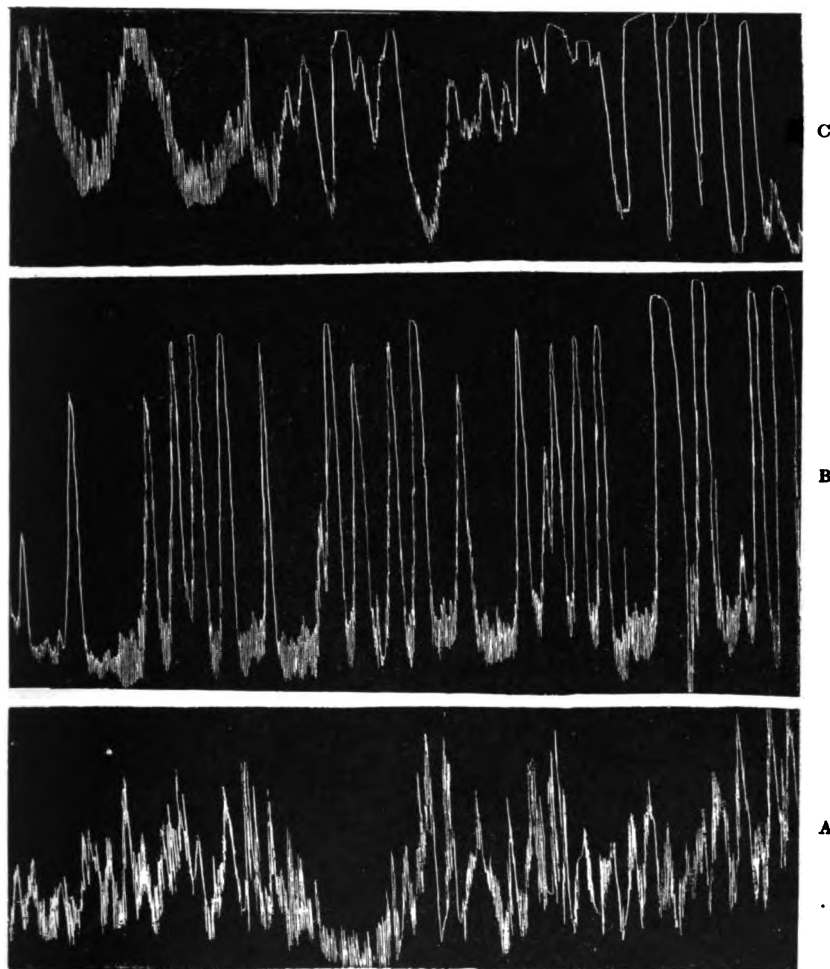


Fig. 1. A, Tracing showing a period of vigorous hunger contractions of the empty stomach of a normal infant. B, Tracing showing exceptionally intense and practically continuous hunger contractions of a 3-months-old infant with persistent pylorus spasm amounting to almost complete pyloric obstruction accompanied by chronic vomiting and gradual loss of weight. C, Tracing showing exceptionally intense hunger contractions and periods of incomplete tetanus of the empty stomach of a five-months-old infant with chronic vomiting ("rumination") and practically stationary body weight. In the right half of the tracing the upward excursion of the manometer had to be checked mechanically to prevent the chloroform from being driven out. Hence the extreme vigor of the gastric contractions are not fully registered. Chloroform manometer. Time, 20 minutes.

by our balloon method as applied to infants. The type of contractions shown by the empty stomach of this infant is shown in figure 1 B. The strength of the contractions was markedly greater than in normal infants. The duration of the periods of contraction was also greater. This indicates a greater than normal gastric tonus. There was no indication of prolonged tetanic contractions.

Case 2¹. Infant 5 months old, chronic vomiting ("rumination"). Practically stationary body weight. Pylorus spasm. A number of observations were made on this infant. When the child was quiet, so that all nervous inhibitory factors were eliminated the empty stomach usually showed the type of contractions reproduced in figure 1 C, that is, hypertonus with periods of tetanic contractions lasting several minutes, interspersed with vigorous contractions of normal duration, an unmistakable condition of hypertonicity and hypermotility. If the infant was asleep during the observation period the tetanic contraction of the stomach invariably caused restless facial grimaces or he would wake up and cry. Such vigorous and prolonged period of tetanic contractions have so far never been observed in the empty stomach of normal infants. They have been observed in adult persons and in dogs after prolonged starvation. This type of contractions of the empty stomach may also be seen in dogs with pancreatic diabetes.

The results on these two infants indicate that pylorus spasm and pyloric stenosis involves either primarily or secondarily a condition of hypertonus and hypermotility of the entire stomach. The excessive contraction of the pylorus may be an expression of this general hypermotility. It is known that the tonus and contractions of stomachs in the young mammal are greater than in the adult and in the old. This may be correlated with the greater tendency to hyperactivity of the pylorus in infancy.

¹ Other details of this case reported by Dr. Strauch, Journ. Am. Med. Asso., 1915, lxx, p. 678.

THE RESISTANCE OF FRESH WATER FISH TO CHANGES OF OSMOTIC AND CHEMICAL CONDITIONS

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The effects of varying the concentration and character of the media in which marine fish live have been the subject of much searching inquiry. Fresh water fish, on the other hand, have been subjected in this connection to only very casual experimentation; P. Bért, (1) for example, investigated the cause of death of fresh water sticklebacks (*Gasterosteus leiurus*) when put into undiluted sea water. In his experiments some individuals lived only two hours, while others survived a month or more—a variability unexplained by him. R. Florentine (2) could not reconcile his results with those of Bért. Although he worked with the same species, caught in a brook near Nancy, he states that no individual fish lived in undiluted sea water more than six hours. Semper (3) states that

The common stickleback, *Gasterosteus aculeatus*, which usually lives in fresh water, lives and thrives perfectly in the Bay of Kiel as well as in the North Sea, and specimens of this fish, caught at Würzburg in the month of May, were even placed at once in sea water without sustaining any injury.

Giard (4) made repeated transfers from fresh to sea water and back, with a daily interval, without perceptibly impairing the vitality of the fish, but he worked with a brackish water stickleback (*Gasterosteus trachurus*). None of these investigations took into consideration the actual quantitative conditions of osmotic pressure, either of the blood of the fish, or of the solutions used. In view of the contradictions and of the fact that the osmotic relationship between the blood and the natural external medium is, for fresh water fish, the reverse of that existing between the blood of marine teleosts and the sea water, it has seemed advisable to experiment with fresh water fish somewhat more systematically than has previously been done.

In selecting material for this work we have purposely avoided possible confusion with the issues of adaptation and adjustment such as natur-

ally arise when working with fish which live in brackish water, or which migrate between fresh and salt water. We have chosen a small hardy form (*Notropis blennius*, Girard),¹ breeding and living throughout the year in the Mississippi and Missouri rivers, several hundred miles from salt water.

Preliminary to these studies the osmotic pressure of the blood of a number of species of Mississippi river fish was determined by the freezing point method (5). The results conformed very closely to those obtained for other species by previous observers; all showed the freezing point of blood of these fresh water fish to be very nearly -0.50°C. , corresponding to an osmotic pressure of approximately six atmospheres. Since the adult fish selected for experimentation are only four to six centimeters in length it was inexpedient to determine the osmotic pressure of their blood directly. Although microscopic examinations of the corpuscles were made after treatment with solutions of various concentrations, the results were not altogether satisfactory, but they led us to believe that we are correct in the assumption that the blood has the same osmotic pressure as that found for all other fresh water teleosts so far investigated. Our experiments strengthen our belief in the correctness of this assumption.

It was found most advantageous to test the lethal action of the solutions. The longevity was determined by placing only two or three fish in a partially covered glass finger bowl containing 100 cc. of the solution. The solution was changed daily. By this procedure control fish were kept without mortality in tap water, at the laboratory temperature, for more than two months. No food was administered during this period, and no fish were experimented with which had not been in the laboratory aquarium at least two weeks.

In the earlier, winter experiments difficulty was experienced in transferring the animals from the cold tap water running into the aquarium (5° – 10°C.): the sudden transfer to a solution or even to tap water at the laboratory temperature (22° – 27°C.) resulted in immediate death of many of the fish (cf. Loeb and Wasteneys) (6). This element of uncertainty was eliminated by allowing the temperature to rise gradually during several hours. The fish were then kept in tap water at the higher temperature for two days before use. Before subjecting them to solutions, they were washed by transferring to distilled water for one-half hour.

¹ I am indebted to Prof. J. F. Abbott for the identification of this fish.

LONGEVITY IN DISTILLED WATER.

Tap water had a distinct saline content; that of St. Louis at the time of these experiments gave a distinct cloudiness due to calcium salts when tested with soluble oxalates, and was strongly alkaline to phenolphthalein. In view of the neutral character of distilled water and in the light of the observations of Ringer (7), (8) concerning the conserving effects, even of small amounts of salts, especially calcium salts, and of alkalis (bicarbonates) it was desirable for purposes of control to determine the longevity in distilled water. In all 31 animals were placed at different times and under variable conditions of temperature, in water redistilled from glass, this distilled water being changed at least daily. Of these, 21 lived, without food, until the experiments were discontinued at which time one fish had lived eight weeks, 8 had survived six weeks, 6 had survived four weeks and 6 had survived three weeks. Of the 10 which did not survive, 4 died in less than twelve hours, death probably being due to lesions which existed prior to the experiment, for they showed distinct abrasions on the body surface especially an inflammatory condition of the tail fin and posterior end of the body.² The other 6 fish died within a week, 2 of these being afflicted with the "gas-bubble disease."

Since 68 per cent of our fish survived the action of redistilled water for weeks and since among the 32 per cent which died several individuals were injured or unhealthy, we can hardly look upon this medium as a very toxic one for this species. Loeb has also shown that among marine fish, the adults as well as the embryos of *Fundulus heteroclitus* live in distilled water. Not even the injured fish of our series were killed with the rapidity reported by Ringer for "minnows, eels, sticklebacks and gold fish," viz., four and one-half to fourteen hours, or by Wells (18) for the white crappie (*Pomoxis annularis*). None the less, within the time limits of our distilled water experiments, a mortality of 32 per cent was never observed among our stock animals which included injured as well as normal individuals; and this is true whether the fish were kept in the tap water aquarium or in finger bowls. These experiments indicate, as did Ringer's, that tap water contains some

² The prompt death of these injured fish is a constant result even in tap water, or in any hypo- or hyper-tonic solution. The fish, however survive much longer in balanced saline solutions isosmotic with the blood and in this respect behave as *Fundulus* has previously been shown to behave, after scraping the body surface; cf. Garrey, (9). Bért (10) had previously noted that eels with an injured surface died when transferred from fresh water to sea water.

substances (salts) which serve to protect the body surfaces (gills) of the fish. They also add to the evidence that there are very marked differences in the vital resistance offered by different species of fresh water fish to distilled water. A further discussion of the effects of distilled water will be found in the section dealing with alkalinity.

THE INJURIOUS EFFECTS OF CANE SUGAR

The fact that a large proportion of the fish lived well in distilled water to which no salts have been added induced us to try out the effects of cane sugar solutions in which it was conceivable that the effects would be purely osmotic, although this conception proved to be erroneous. The sugar was twice recrystallized in two experiments; in eight others, "highest purity" (Merck) sugar was used and in two series of experiments commercial "Domino" sugar was used. Little difference could be noted in longevity in these different solutions although on the whole the fish lived a negligibly longer time (3-12 hours) in the commercial product. The results of the experiments are summarized in Table I.

TABLE I

MOLAR CONCENTRATION	$\Delta = C^{\circ}$	PER CENT DEAD AT END OF			
		12 hrs.	24 hrs.	2 days	3 days
0.1	-0.198	0	33	100	
0.2	-0.397	0	0	33	100
0.25	-0.49	0	33	66	100
0.3	-0.59	33	100		
0.4	-0.77	100			

These experiments demonstrate clearly that the fish die within a few hours (12) in a 0.4 molecular cane sugar solution which has an osmotic pressure about 50 per cent above that of their blood, but live much longer (3 days) in a solution of approximately the osmotic pressure of the blood, e.g., between 0.2 and 0.25 mol.

While the influence of osmotic pressure is clearly demonstrated in the increased mortality of the fish in solutions with concentrations above that of their blood, other factors were clearly involved, for the fish died sooner in $\frac{M}{10}$ cane sugar solutions than in either distilled water or 0.2 mol. solutions of cane sugar.

The results tabulated above clearly show that cane sugar solutions are, either directly or indirectly, toxic. This toxicity can be decreased

by increasing the osmotic pressure of any hypo-osmotic solution of this sugar until it is equal to that of the blood, but above this concentration, which seems to be optimum, a further increase in the osmotic pressure increases, progressively, the toxicity of the solution.

Bért ascribed the effects of changing the concentration of media to which fish were subjected, to a primary injury to the gills, as he stated it, "a physico-chemical alteration in the properties of the (gill) membranes." He also described a "very congested" condition of the gills due to the action of sea water. Ringer, Loeb, and also Herbst (11) point out the probability of a change in permeability of the gills as the event of primary importance. J. Loeb (12) has more recently attributed the increased permeability of the gills of *Fundulus* in sugar solutions, in part at least, to fermentative acid formation. That acid is a factor he demonstrated conclusively, but he also observed a direct toxic action of the sugar solutions. His experiments demonstrated a greater death rate in cane sugar than in isotonic dextrose solutions, which we believe would be difficult to understand if the only factor at work was that of acidity. The direct toxic action of sugar in experiments with *Fundulus* was noted by Loeb in solutions more concentrated than $\frac{M}{4}$. This concentration is hypertonic for fresh water fish. As a result, possibly of the membrane changes due to the action of hypertonic sugar solutions upon the gills, the osmotic extraction of water doubtless becomes a factor in increasing the toxic effects (even for *Fundulus*). That the mortality in solutions of cane sugar is, however, not primarily an osmotic effect, but must be referred to some other mechanism is demonstrated in our experiments by the fact that the fish live much longer in certain saline mixtures of equivalent or even greater osmotic pressures. Similarly, taking advantage of the fact which Loeb found, in this connection, viz., a distinct antagonism of mixtures of salts for the toxic effects of cane sugar solutions, we have found that in a mixture of equal parts of $\frac{M}{4}$ cane sugar and a weak Ringer's solution ($\Delta = 0.46^\circ\text{C}.$) the fish lived more than two weeks, while in $\frac{M}{4}$ cane sugar solution which therefore had the same sugar content, but only half the osmotic pressure of the mixture, the fish did not survive the third day. All solutions were freshly made and changed daily.

In the action of cane sugar upon these fresh water fish, our results deviate in only one particular from those obtained by Loeb for the marine form, *Fundulus*, viz., the greater toxicity of weaker than of the more concentrated sugar solutions when both are hypotonic. It seems hardly plausible to attribute this result to a greater acidity of

the weaker solutions or to a lower toxicity of the more concentrated solutions. Leo Loeb (22) has described marked changes in the surface and cytoplasm of blood cells of *Limulus* due to sugars. In the case of fish the toxic factors would seem to be such as to increase the permeability of the surface or gill membranes and thus to introduce the play of osmotic forces which previously had been ineffective. In consequence the tissues and body fluids of the fish take up water from hypotonic solutions, the more dilute the more rapidly, but are spared the rapid dilution (and possibly the loss of salts) by increasing the concentration of the external medium. It should be added that the experimental results exclude hyperglycemia (saccharosemia) as the cause of the toxic action of cane sugar.

THE TOXICITY OF DILUTED SEA WATER

The experiments of Bért indicated clearly that, had it been used in proper concentration, sea water would not have been very toxic for fresh water fish, and it has since been amply demonstrated that for tissues of a great variety of forms diluted sea water must be looked upon as a very well "balanced solution" (13). It was therefore deemed probable that it could be used as a control with which to check up the osmotic action of other solutions. The effects of various dilutions of sea water upon the longevity of our fresh water fish are shown in Table II.

TABLE II

CONCENTRATION		$\Delta = C^{\circ}$	LONGEVITY
1	Undiluted sea-water (Woods Hole)	-1.81	20 minutes or less
2	75 cc. sea-water + 25 H ₂ O (dist.)	-1.35	20 minutes
3	60 cc. sea-water + 40 H ₂ O (dist.)	-1.09	25 minutes to 40 minutes
4	50 cc. sea-water + 50 H ₂ O (dist.)	-0.915	1 hr. 5 min. to 1 hr. 45 min. (average 1 hr. 15 min.)
5	45 cc. sea-water + 55 H ₂ O (dist.)	-0.82	2 hrs. to 6 hrs. (average 4 hrs.)
6	40 cc. sea-water + 60 H ₂ O (dist.)	-0.73	Min. 4 hrs., max. 20 hrs. (average 12 hrs.)
7	35 cc. sea-water + 65 H ₂ O (dist.)	-0.64	Average 2 weeks, max. 5 weeks
8	30 cc. sea-water + 70 H ₂ O (dist.)	-0.547	6 weeks—experiments discontinued.
9	In all more dilute sea-water the fish lived as well as in tap water.		

The solution containing 30 cc. sea water diluted with 70 cc. of distilled water has a freezing point of 0.547°C. and is only very slightly hypertonic for the blood. At this concentration the curve of longevity takes a sharp turn. The isotonic sea water was not more detrimental than the more dilute sea water or than tap water, and less harmful than distilled water under the best of laboratory conditions. An

isotonic solution may thus be regarded as having the "critical" concentration, since more concentrated solutions were rapidly and progressively more detrimental as the concentration increased. Bért went so far as to state that the sole cause of death of *frogs* in sea water is attributable to the desiccation of blood and tissues. He noted a loss of weight by fish as well as by frogs which amounted to $\frac{1}{4}$ or even $\frac{1}{2}$ of the body weight; Loeb and Wasteneys (14) have recently added some interesting data on the osmotic changes of *Fundulus* in various solutions.

We have demonstrated that in the case of hypotonic solutions the mixture of salts in the proportion in which they are found in sea water is non-toxic for the fresh water fish with which we have experimented, and that the deleterious effects of the hypertonic solutions are mainly osmotic and proportional to their water extracting proclivities.

The lack of resistance of our fresh water fish, when placed in sea water which is hypertonic for their blood, is in interesting contrast to the resistance which marine teleosts exhibit, for they normally are not affected by the hyperosmotic sea water in which they live.

THE TOXICITY OF SODIUM CHLORIDE

In striking contrast to the longevity of the fish in diluted sea water is their mortality in weak solutions of sodium chloride as shown in Table III.

TABLE III

PER CENT NaCl	$\Delta = ^\circ\text{C}.$	LONGEVITY			COMPARATIVE LONGEVITY IN SEA WATER OF SAME OSMOTIC PRESSURE
		Maximum	Minimum	Average	
1.35	-0.8	1 hr. 46 min.	50 min.	1 hr. 15	2 hrs.
0.9	-0.54	3 days	30 hrs.	2 days	14 days
0.76	-0.46	15 days	3 days	4 days	6 weeks+
0.6	-0.37	15 days	4 days	12 days	6 weeks+
0.45	-0.275	23 days		15 days	6 weeks+
0.25	-0.105	24 days	9 days	18 days	6 weeks+

It was noted above (Table II) that in sea water iso-osmotic with the blood of these fish, and in all more dilute sea water, the experimental animals survived the duration of the experiment (6 weeks). Table III shows that in sodium chloride of this concentration (0.76 per cent) they did not survive a week. Although little difference is to be noted

between the toxicity of 0.45 per cent and 0.25 per cent solutions of sodium chloride, there is a progressive increase in the toxicity with increasing concentration, until, at that of the blood, i.e., at the "critical concentration," the toxicity curve takes a sudden turn and the mortality of the animals increases at a very rapid rate. The slow increase in mortality with the increase in the concentration of the hypotonic solutions is very nearly proportionate to the mass action of the sodium chloride, but the change in the mortality curve shows that a new lethal factor has been introduced at the "critical concentration." This factor is superimposed upon the toxic action of the salt (NaCl), and is, we believe, osmotic in character. This view is strengthened by the fact that with the stronger (hypertonic) solutions the differences between the toxicity of NaCl and of sea water are not striking; although sodium chloride was on the average the more toxic, the variability in the longevity of individual animals was such as to make any quantitative statement of this conclusion unsafe.

The non-toxic character of hypotonic sea water—a "balanced solution"—as contrasted with the striking toxicity of equivalent concentrations of sodium chloride, pointed to these fish as especially favorable material for the study of the antagonistic action of salts, and work was extended along these lines.

CALCIUM CHLORIDE

We will record first a summary of the results obtained with pure solutions of calcium chloride.

- In CaCl_2 ($\frac{M}{20}$) the fish lived only 3.5–6 hours.
- In 50 cc. CaCl_2 ($\frac{M}{20}$) + 50 cc. H_2O (dist.) the fish lived 2–4 days.
- In 25 cc. CaCl_2 ($\frac{M}{20}$) + 75 cc. H_2O (dist.) the fish lived 4–20 days.
- In 15 cc. CaCl_2 ($\frac{M}{20}$) + 85 cc. H_2O (dist.) the fish lived 14–21 days.
- In 5 cc. CaCl_2 ($\frac{M}{20}$) + 95 cc. H_2O (dist.) the fish lived 5–7 weeks (exp. discontinued).

In this series of experiments the calcium chloride was found to be much more toxic than sodium chloride of the same concentration; for example, the longevity in $\frac{M}{20}$ CaCl_2 (2–4 days) is not greater than in $\frac{M}{8}$ NaCl in spite of the higher osmotic pressure of the latter solution.

MIXTURES OF SODIUM AND CALCIUM CHLORIDE

The well-known antagonism of calcium salts for the toxic effects of sodium chloride was forcibly illustrated in experiments of which the following series is typical.

67 cc. NaCl (1.35 per cent) + distilled water to 100 cc. (=0.9 per cent NaCl) fish lived 2 days.

67 cc. NaCl (1.35 per cent) + 0.5 cc. CaCl_2 ($\frac{M}{10}$) + dist. H_2O to 100 cc. fish lived 4-8 days.

67 cc. NaCl (1.35 per cent) + 1.0 cc. CaCl_2 ($\frac{M}{10}$) + dist. H_2O to 100 cc. fish lived 8-10 days.

67 cc. NaCl (1.35 per cent) + 5.0 cc. CaCl_2 ($\frac{M}{10}$) + dist. H_2O to 100 cc. fish lived 8-10 days.

Adding 10, 15, 20 and 25 cc. of CaCl_2 ($\frac{M}{10}$) to the 0.9 per cent sodium chloride did not produce any more favorable results, nor did the presence of these larger amounts of calcium perceptibly increase the toxicity. The experiment illustrates how little calcium is necessary to produce a marked decrease in the toxicity of sodium chloride. The reciprocal antagonism of the salts of sodium for those of calcium is also revealed, for experiment showed that the fish live 8 or 10 days in a mixture of 75 cc. NaCl (0.9 per cent) + 25 cc. CaCl_2 ($\frac{M}{10}$). This solution contains $\frac{M}{40}$ CaCl_2 , in pure solutions of which the fish lived only 2-4 days. Sodium chloride, thus, decreased the toxicity of the CaCl_2 . The mixture of these two salts is not, however, as favorable a medium for these fish as is sea water of the same concentration. In the hope of determining the rôle of the other salts in producing the physiological balance of sea water, the experiments were continued and the toxicity of the salts and their mixtures in various proportions was tested.

POTASSIUM CHLORIDE

Solutions of pure potassium chloride proved more toxic than similar solutions of the equi-osmotic solutions of the chlorides of either sodium or calcium, as is seen in the following experiment.

KCl ($\frac{M}{10}$)	the fish died in 3-5 hrs.
KCl ($\frac{M}{10}$) 50 cc. + H_2O distilled, 50 cc.	the fish died in 3-8 hrs.
KCl ($\frac{M}{10}$) 25 cc. + H_2O distilled, 75 cc.	the fish died in 12-18 hrs.
KCl ($\frac{M}{10}$) 15 cc. + H_2O distilled, 85 cc.	the fish died in 20 hrs.
KCl ($\frac{M}{10}$) 5 cc. + H_2O distilled, 95 cc.	the fish died in 12-29 hrs.

MIXTURE OF NaCl + KCl

In a mixture of 95 cc. $\frac{M}{10}$ NaCl + 5 cc. $\frac{M}{10}$ KCl the fish lived two or three days. Here again, we find that sodium chloride has in some measure served to protect the animals from the toxic action of KCl, just as it did from CaCl_2 , a result which is evident in spite of the fact

that the osmotic pressure was practically at the limit of the animals' tolerance.

The reciprocal action by which the toxicity of sodium chloride would be decreased by potassium chloride was not revealed in our experiments as it was in those of Loeb and Wasteney (15) with *Fundulus*. Our animals did not live longer in any mixture of sodium and potassium chloride than in pure sodium chloride of the same osmotic pressure. The addition of progressively increasing amounts of potassium chloride from 1 cc. to 25 cc. per 100 cc. of the mixture caused a progressive shortening of the period of survival of the fish. It is possible that we were working with concentrations of NaCl which were too great and that success in this direction may attend a different mode of attack.

MIXTURES OF POTASSIUM CHLORIDE AND CALCIUM CHLORIDE

Calcium chloride reduces markedly the toxicity of potassium chloride for these fish, as is illustrated in the following series of experiments.

5 cc. KCl ($\frac{M}{10}$) + 95 cc. H ₂ O distilled	Longevity 24 hrs. (average).
5 cc. KCl ($\frac{M}{10}$) + 1 cc. CaCl ₂ ($\frac{M}{10}$) + H ₂ O distilled to 100 cc.	Longevity 6 days 1 lived 13 days).
5 cc. KCl ($\frac{M}{10}$) + 15 cc. CaCl ₂ ($\frac{M}{10}$) + H ₂ O distilled to 100 cc.	Longevity 9 days.
15 cc. KCl ($\frac{M}{10}$) + 15 cc. CaCl ₂ ($\frac{M}{10}$) + H ₂ O distilled to 100 cc.	Longevity 13 days
15 cc. KCl ($\frac{M}{10}$) + 0	+ H ₂ O distilled to 100 cc. Longevity 20 hrs.
25 cc. KCl ($\frac{M}{10}$) + 0	+ H ₂ O distilled to 100 cc. Longevity 12-18 hrs.
25 cc. KCl ($\frac{M}{10}$) + 5 cc. CaCl ₂ ($\frac{M}{10}$) + H ₂ O distilled to 100 cc.	Longevity 36 hours.
25 cc. KCl ($\frac{M}{10}$) + 15 cc. CaCl ₂ ($\frac{M}{10}$) + H ₂ O distilled to 100 cc.	Longevity 36 hours.
25 cc. KCl ($\frac{M}{10}$) + 25 cc. CaCl ₂ ($\frac{M}{10}$) + distilled to 100 cc.	Longevity aver. 36 hours (one animal lived 7 days).

In these experiments, as in those with sodium chloride, the decrease in toxicity of KCl is demonstrable even with very small amounts of calcium chloride but the criterion, longevity, does not warrant any interpretation which would fix an optimal ratio for these mixtures. On the other hand, we were not able to affirm from our experiments that there was a decrease in the toxicity of solutions of calcium chloride by the addition of KCl. In all of a large number of experiments to test this point, experiments with a comprehensive range of concentration of both calcium and potassium solutions, the presence of potassium chloride invariably caused death of the fresh water fish sooner than the control solutions containing calcium chloride alone.

Summarizing the results of our experiments with KCl, we may say that for these fresh water fish this salt is more toxic, molecule for

molecule, than either NaCl or CaCl₂. Both Na and Ca materially decreased the toxicity of potassium chloride. The reciprocal detoxication by this salt of either Na or Ca was not sufficiently striking, if present, to admit of demonstration by these experiments.

No fact has been more firmly established by experiment than that for a large variety of tissues and organisms, the addition of potassium chloride to mixtures of Na and Ca is favorable to life and function. Further experimentation showed that this can also be demonstrated for *Notropis blennius* in spite of the contradictory character of the results obtained in the experiments with sodium chloride or calcium chloride alone when KCl was added. It has already been noted that in pure 0.9 per cent NaCl the fish lived only two days at the most. The addition of CaCl₂ to the sodium chloride prolonged the life to a maximum of ten days. Fish were at the same time placed in a parallel series containing sodium chloride (0.9 per cent), but to the solution was added CaCl₂ (ad .024 per cent) and KCl (ad .042 per cent). This is the proportion which is frequently used in making "Ringer's solution" for experiments with mammalian tissues. The solution had a $\Delta = -0.55^{\circ}\text{C}$. In this solution the minimal duration of life of our fresh water fish was equal to the maximum in sodium chloride solutions with the optimal amount of calcium chloride, while the longest duration of life in this solution was double that in the mixture which contained the other two salts but did not contain the potassium chloride. In passing it may be noted that the amount of potassium chloride in this solution if present in distilled water alone, causes the death of these fish in 24 hours, and that we have here an excellent example of the neutralization of the toxicity of potassium by a mixture of Na and Ca as well as of the fact that the Na-Ca complex is decreased in toxicity by a substance in itself more toxic than the complex, and further that the effect upon the Na-Ca complex is demonstrable even though we could not demonstrate an antagonistic effect upon the individual chemicals of the mixture.

Ringer's solution, slightly alkaline, gives a good example of what is meant by physiological "balance," i.e., *mutual* decrease of toxicity. We know of no tissue for which Ringer's solution is not less toxic than pure sodium chloride solution; it is not, however, the *best* saline solution for the fresh water fish with which we experimented—it is not perfectly balanced. The earlier experiments of our series showed that healthy individuals lived in sea water of the same osmotic pressure as the Ringer's solution (30 cc. of Woods Hole sea water + 70 cc. distilled

water) for six or seven weeks, at the expiration of which time the experiments were discontinued. Out of many individuals tested not one lived more than four weeks in the Ringer's solution and few survived more than two weeks.

THE EFFECTS OF MAGNESIUM CHLORIDE

In attempting to determine why sea water was less toxic than Ringer's fluid, our attention is naturally directed to the large content of magnesium salts which are not present in Ringer's fluid. Almost exactly one-tenth of the molecules of sea water are of magnesium salts (van't Hoff) their concentration is therefore equal to $\frac{M}{10}$ $MgCl_2$. Bért and DeVarigny have both contended that magnesium salts in the concentration in which they are present in sea water are not toxic for fresh water sticklebacks. This is surprising in the face of such results as the following which we obtained with *Notropis blennius*.³

- (a) $MgCl_2$ ($\frac{M}{10}$) Average longevity 1 hour 10 minutes.
- (b) $MgCl_2$ ($\frac{M}{10}$) 50 cc. + distilled H_2O 50 cc. Average longevity 20 hours-24 hours.
- (c) $MgCl_2$ ($\frac{M}{10}$) 25 cc. + distilled H_2O 75 cc. Average longevity 48 hours.
- (d) $MgCl_2$ ($\frac{M}{10}$) 15 cc. + distilled 85 cc. Average longevity 3-5 days.
- (e) $MgCl_2$ ($\frac{M}{10}$) 5 cc. + distilled H_2O 95 cc. Average longevity 4-6 days.

Solution (a) has approximately the osmotic pressure of the blood of the fish; solution (b) has about the concentration of this salt in sea water ($\frac{M}{10}$); solution (d) in which the fish live five days at most contains the same content of magnesium chloride ($\frac{M}{10}$) as 30 cc. of sea water diluted with 70 cc. of distilled water in which the fish lived six weeks or more. Obviously magnesium chloride is very toxic, but its toxicity is reduced to a minimum by the presence of the other salts of diluted sea water; conversely we may infer that magnesium decreases the toxicity even of so well balanced a mixture of Na, Ca and K chloride as Ringer's solution.

An objection may be raised to these conclusions, viz., that the presence, in sea water, of anions not present in Ringer's solution, might account for the lower toxicity of the sea water and that the effect

³ It is worthy of note that the fish in solutions of magnesium chloride show distinct anaesthetic effects for some time before death. They are unable to maintain their equilibrium, lie upon the side or with ventral surface up when at rest, and roll about the long axis when swimming. Respiratory movements of the gill opercula are of the Cheyne-Stokes type.

may not be due to magnesium. To obviate this criticism mixtures of chlorides alone were compared, all in $\frac{M}{10}$ concentration. (a) Ringer's fluid: NaCl, 100 cc. + KCl, 2.2 cc. + CaCl_2 , 2.0 cc. In this solution the fish lived about two weeks. (b) Ringer's fluid as in solution (a) + 12 cc. MgCl_2 . In this solution the fish were still alive at the end of five weeks whereupon the experiment was discontinued. The magnesium chloride then antagonized the toxicity of the neutral Ringer's fluid to a marked degree although the concentration of magnesium was such that in the absence of the other salts, it would have killed the fish in four or five days. The above criticism then is not valid, and the antagonism noted with sea water was due to the presence of magnesium salts.

Loeb has demonstrated, unequivocally, the antagonistic action of magnesium salts for the toxic action of sodium salts. We were astonished to find that no such antagonism was demonstrable in our series of experiments directed to this end. In fact the addition of MgCl_2 to NaCl always increased the toxicity of the solution, acting in this respect like KCl. The contrast between this failure and the marked conserving action exerted by magnesium salts upon the mixtures of Na + K + Ca chloride would seem to point to an antagonism which must be referred to an effect exerted in the main to decrease the toxicity either of calcium or of potassium, or of their combined effect—which may be different from that of either salt singly.

MIXTURES OF MAGNESIUM CHLORIDE WITH POTASSIUM CHLORIDE

A very distinct antagonism of MgCl_2 for $\frac{M}{10}$ KCl was observed as is illustrated in the following series. (a) In 15 cc. KCl ($\frac{M}{10}$) + 85 cc. distilled water the average longevity was 20 hours and the maximal 48 hours (one fish only). (b) In 15 cc. KCl $\frac{M}{10}$ + 80 cc. H_2O + 5 cc. MgCl_2 ($\frac{M}{10}$) the minimal duration of life was 70 hours and the maximal between 5 and 6 days, showing the conserving action of the magnesium salt. (c) In the same concentration of potassium chloride but with 15 cc. of MgCl_2 ($\frac{M}{10}$) the first death occurred in 28 hours, the average longevity was 72 hours and the maximal just under four days. The fish still lived longer than in the pure solution of KCl, but not so long as with the smaller amount of magnesium chloride. Increasing the content of the magnesium salt above that in solution c) caused a progressive increase in toxicity. (d) When the solution contained 25 cc. of MgCl_2 ($\frac{M}{10}$) the fish lived only two or three days, but the solution was

less toxic than the pure KCl solution without Mg. The solution was more toxic than one containing magnesium chloride alone, showing that the toxicity of the magnesium salt had not been decreased by the presence of KCl in any of the concentrations used.

MAGNESIUM VS. CALCIUM

Loeb (16) demonstrated that the continued contraction of the muscles of *Polyorchis*, which appears in solutions containing calcium, is antagonised by magnesium with resultant relaxation; and Meltzer and Auer (17) showed that the anaesthetic action of magnesium sulphate was neutralized by calcium chloride. Concerning these reciprocal antagonisms, the following data were obtained with mixtures of $\frac{M}{15}$ solutions of the chlorides of magnesium and calcium.

15 cc. MgCl ₂	+85 cc. H ₂ O	Lived 4 days.
15 cc. MgCl ₂ + 5 cc. CaCl ₂	+ 80 cc. H ₂ O	Lived 5-7 days
15 cc. MgCl ₂ + 15 cc. CaCl ₂	+ 70 cc. H ₂ O	Lived 6-7 days.
25 cc. MgCl ₂ + 0 cc. CaCl ₂	+ 75 cc. H ₂ O	Lived 2 days.
25 cc. MgCl ₂ + 10 cc. CaCl ₂	+ 65 cc. H ₂ O	Lived 6 days.
25 cc. MgCl ₂ + 25 cc. CaCl ₂	+ 50 cc. H ₂ O	Lived 6 days.
25 cc. CaCl ₂	+ 75 cc. H ₂ O	Lived 4-6 days.

Calcium chloride unquestionably decreased the toxicity of magnesium chloride. Concerning the reciprocal relation of Mg to Ca: the presence of magnesium at least did not increase the toxicity of the calcium chloride although the osmotic pressure was raised by its addition, and in the last figures given there is an indication of some decrease in the toxicity of CaCl₂ due to the addition of MgCl₂.

ALKALINITY

Some of the facts which have developed in the course of this investigation are not without significance in view of the recent generalization of Wells (18) that "fresh water fish cannot live normally in water that is alkaline but require a certain degree of acidity to carry out their normal activities." The fish with which we have worked have been kept in our laboratory for months in the running tap water; they and many other species are similarly kept in the aquaria of the dealers in fish bait, and in other aquaria about the city of St. Louis. The city water of St. Louis is quite strongly alkaline owing to the method of purification of the Mississippi river water by precipitation with lime and ferrous sulphate. It gives an alkaline reaction to phenolphthalein,

rosolic acid, neutral red, congo red, methyl violet and litmus. It shows a distinct titratable alkalinity as shown by the reports of the chemist. Sørensen's colorimetric methods in our hands showed the concentration of OH^- to be somewhat more than $N \times 10^{-5}$.

In aquaria plentifully supplied with this running water the alkalinity was not perceptibly altered by the presence of the fish; neither the body excretions nor CO_2 developed by the fish had any effect in decreasing the alkalinity of the aquarium water.

The fish lived for weeks in all dilutions of sea water less concentrated than their blood. Woods Hole sea water was used and this has been found by Loeb (19) to have an alkalinity somewhat greater than $\text{OH}^- \times 10^{-5}N$. The stock Ringer's solution of our laboratory contains 0.03 per cent sodium bicarbonate and we have noted a minimum longevity of two weeks in this solution. This solution was rendered much less toxic for our fish by the addition of magnesium chloride which, in this saline mixture, did not alter the hydroxyl ion content.

The presence of neutral salts (properly balanced) in the above solutions may have decreased the toxicity of the hydroxyl ions; while this possibility should be further investigated, Ringer's (7) experiments, which we have confirmed, showed that bicarbonate of sodium (0.03 per cent) alone decreased the toxicity of distilled water for fresh water fish.

As to the necessity of acidity, the above experiments speak conclusively against its requirement by the species of fish with which we worked. Of further evidence in this direction it should be noted that the animals could be kept for considerable periods in distilled water and much longer in balanced mixtures of the neutral chlorides. The only possibility of acid development in these solutions lies in the accumulation of CO_2 excreted by the animals themselves. This accumulation of course, cannot be entirely eliminated by the finger bowl method; it was reduced to a minimum, however, by frequent changes of water and by the free access to the air and agitation which was obtained in all experiments by the movements of the fish, and in some by bubbling alkali-washed air through the solutions. In none of the experiments was the sea water or alkaline Ringer's fluid rendered acid by any such accumulation of CO_2 . Finally experiments were made in which distilled water and neutral salt solutions were changed by a continuous stream and constant level device so that double the volume of the fluid was replaced each hour. The animals were alive at the end of three weeks when the experiments were discontinued.

Alkali in the form of sodium bicarbonate was found by Wolfgang Ostwald (20) to prolong the life of fresh water *Gammarus* when placed in saline solutions and our experience indicates that this is true also for the fresh water fish (*Notropis blennius*). This result was to be expected for Gaule (21) in 1878 showed that alkali was necessary to prevent the development of acidity by the beating heart. Since that time the presence of a certain excess of free $\overline{\text{OH}}$ ions along with available alkalinity has been found to be advantageous to the functional activity of tissues by all physiologists who have worked with conservative fluids (Ringer, Langendorf, Locke, Tyrode, Clark).

That differences in resistance of various species of fresh water fish to alkalis should be found is not surprising, similar differences have been noted by J. Loeb (19) between such closely related marine forms as *Strongylocentrotus purpuratus* and *Arbacia punctulata*. The eggs of the former begin development only in the presence of alkali, while those of the latter will begin but not continue development in neutral or even faintly acid solutions; even for the latter the optimal fluid contains free OH ions in the concentration in which they are present in Woods Hole sea water ($C_{\text{OH}} = >N \times 10^{-5}$.)

On the whole it may be said that, except for osmotic and concentrational differences, the resistance of fresh water fish to salts and their mixtures is quite like that of marine fish. It therefore seems futile to attribute the experimental results with either form to special adaptation or adjustment.

SUMMARY

1. The fresh water fish, *Notropis blennius*, will live months in the tap water of St. Louis, which is distinctly alkaline. These animals also live in redistilled water for weeks.

2. For these fish cane sugar solutions are directly toxic, least so if the osmotic pressure is equal to that of the blood. This toxicity is reduced by salts and alkalis.

3. Individually the solutions of chlorides of potassium, magnesium calcium and sodium are toxic. In equivalent concentrations the relative toxicity is $K > Mg > Ca > Na$.

4. Sodium chloride decreased the toxicity of chlorides of Ca, Mg and K. Calcium chloride decreased the toxicity of chlorides of Na, K and Mg. Magnesium chloride decreased the toxicity of chlorides of Ca and K but not that of sodium. Potassium chloride did not decrease the toxicity of any single salt (Ca, Na or Mg).

5. In combinations of two or more of the other salts the antagonistic decrease of toxicity by K and by Mg was apparent. Thus mixtures of the salts demonstrated their mutual antagonism and became progressively less toxic when to sodium chloride was added calcium chloride then potassium chloride and finally magnesium chloride.

6. Sea water diluted to the concentration of the blood (or less) was a perfectly balanced solution for the fresh water fish.

7. When the salts are properly balanced the fresh water fish tolerated an osmotic pressure of the external medium equal to their own blood. Above this concentration, which is "critical," death is prompt.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE EMPTY STOMACH

XXXII. THE EFFECT OF DREAMING ON THE GASTRIC HUNGER CONTRACTIONS

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During the course of experiments on the movements of the empty stomach of dogs I have noted on several occasions an inhibition of the gastric hunger contractions brought about probably by cerebral influences in the dreaming state during deep sleep. These observations I wish to record in a brief note chiefly because they show that even during sleep emotional states (whether pleasant or unpleasant) may influence bodily states.

The author does not assume that such or similar observations have not been made before. Most of us have experienced so-called night mares with the result that we have awakened ourselves from sleep by our own cry of despair or fright. Other dreams lead to the speaking of a few coherent or incoherent sentences. Or, as the result of emotional stress brought on by pleasant or unpleasant dreams, persons break out into a fit of laughter or wake up weeping. It would seem that the nature of the emotion effects the same bodily change or leads to the same bodily activity irrespective of whether the body is in a waking or sleeping state.

Every form of afferent stimulus in the dog whether agreeable (sight of food, sight of a favorite attendant) or disagreeable (pain, sight of a natural enemy as the cat) leads promptly in the waking state to a diminution in tone of the empty stomach or temporary cessation of the hunger contractions.¹ Pleasure, pain, or fear leading to diminished activity of the empty stomach in the waking state, the same emotional state occurring during sleep might lead to the same inhibition.

It is a simple matter to determine whether or not a dog is asleep. It is more difficult to be certain that dogs dream in the sleeping state.

¹ Carlson: This Journal, 1913, xxxii, 369.

If the dogs, after lying in my lap for some time at ease and amid perfectly quiet surroundings with closed eyes and a regular and slow or slow and stertorous respiration (snoring) would suddenly begin to wag tail, breath more deeply and irregularly, move more or less coördinately fore and hind limbs, show teeth, snarl, or emit abortive yelps, I assumed that the dog was experiencing during sleep a form of cerebral excitation akin to or identical with the dreaming state in man.

METHODS

The method used to obtain contractions of the empty stomach was the same as used in all previous experiments recorded in this series of papers.²

RESULTS

Three figures will suffice to show graphically the effect of cerebral excitement during the sleeping state on the contractions of the empty stomach in dogs.

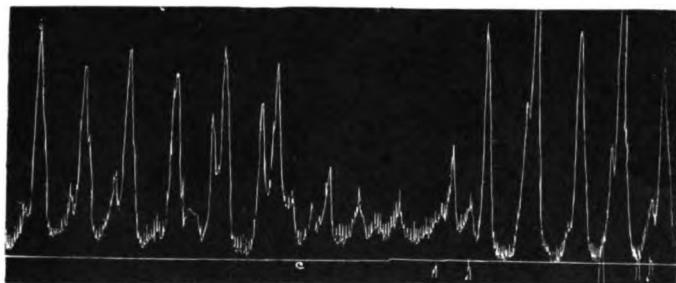


Fig. 1.

Figure 1. During the entire experiment the dog lay in my lap asleep. For $\frac{3}{4}$ of an hour dog showed Type II contractions as shown prior to "c" on the tracing. At "c" dog's hind legs twitched and jerked about incoördinately. This continued for some time. As can be seen from the tracing there is almost a complete cessation of the stomach contractions which persists for some time before the regular Type II rhythm reappears.

Figure 2. Prior to "a" the dog was in my lap sleeping peacefully. As can be seen from the tracing the contractions of the empty stomach

² Carlson: This Journal, 1913, xxxii, 369.

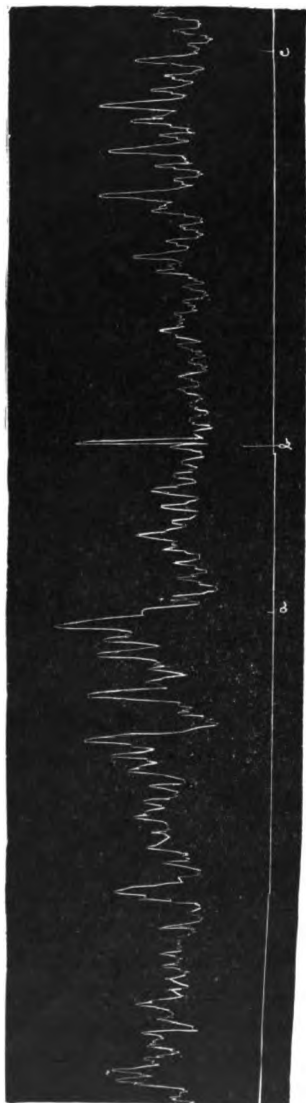


Fig. 2.

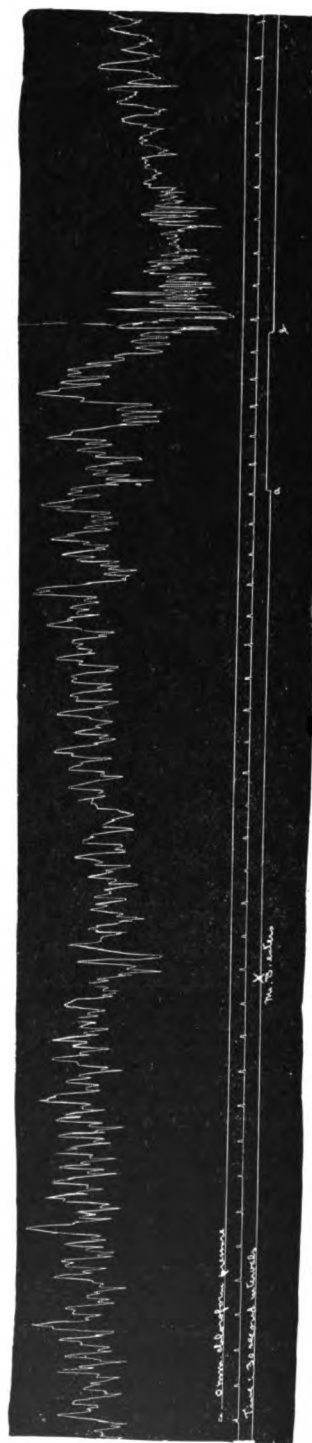


Fig. 3.

were increasing in vigor. There now occurred irregular twitchings of the toes and other muscles of the fore and hind limbs. As a result of these movements the more vigorous type of stomach contractions ceased. The dog woke up at "b" stretched herself and with the beginning of the purely sleeping state the stomach contractions gradually increased in vigor.

Figure 3. Dog sleeping in my lap showed vigorous type III hunger contractions. The tone of the empty stomach was temporarily inhibited at "x" when Mr. B. entered the room. The dog fell asleep and the tone of the stomach returned. Shortly before "a" on the tracing the respirations were deepened. From "a"—"b" the facial muscles contracted spasmodically, the feet moved, and the respiration was increased in depth. At "b" dog woke up with a start and this was followed by a complete cessation of the movements of the empty stomach which lasted for some time. It can be seen, however, that even before awakening the tone of the stomach was gradually diminishing.

DISCUSSION

Since the sensation of hunger, even when painful, does not itself inhibit further movements of the empty stomach the impulses which give rise to this inhibition must have their origin in some organ other than the stomach. The inhibitory impulses probably reach the stomach through the splanchnic nerves, the impulses having their origin in the central nervous system as one of the end effects of the emotional state. There is no evidence to support the view that the inhibition is brought about by the direct action of the splanchnic nerves on the stomach musculature or due to the increased output of adrenalin as Cannon and D. de la Paz have shown to be the cause of the inhibition of the movements of the stomach during digestion by fear, pain, rage.³ The rapidity in the appearance of the inhibition points to nervous rather than to a chemical inhibition. It is possible but not probable that the inhibition results from afferent impulses arising from stimulation of the sensory nerve endings in the muscles, skin, and joints as a secondary effect of the active movements of the facial muscles and muscles of the extremities thrown into activity by impulses coming to them from the cerebrum.

³Cannon and de la Paz: This Journal, 1911, xxviii, 64.

SUMMARY

Although the contractions of the empty stomach (hunger contractions) become more vigorous with the onset of and during sleep because the inhibitory mechanism is no longer influenced by the sensory impulses reaching it through the optic, olfactory, and acoustic nerve (the extero-ceptive field of Sherrington) the hunger contractions may be greatly diminished even during sleep if the sleeping state is accompanied by pronounced cerebral activity (dreaming). The same cerebral state which effects movements of the limbs, tail, and muscles of the face effects an inhibition of the contractions of the stomach. The inhibition is therefore purely central in origin since it is not brought about by any impulse coming into the central nervous system by any known sensory nerve.

THE MODE OF ACTION OF ULTRA-VIOLET RADIATION IN INJURING LIVING CELLS WITH SPECIAL REFER- ENCE TO THOSE CONSTITUTING THE EYE

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Action on paramecia. Five cc. of water containing paramecia were introduced into a glass vessel 6 cm. in diameter and 4 cm. deep. This vessel was adjusted partially immersed in running water under a quartz mercury-vapor burner operating at 140 volts, 3.3 amperes, 2400 cp. so that the burner was 10 cm. from the surface of the liquid. The organisms were observed under a microscope during the exposure. These organisms are fairly transparent and appear to the unaided eye as white specks in the water. During the exposure the animals moved more and more slowly and gradually became granular and opaque. After 30 or 40 minutes they were dead. Figure I (1) represents the normal transparent animal, (2) represents the organism that was killed by ultra-violet radiation and (3) one killed by heating to 45°C. Just as exposure of egg white to ultra-violet radiation coagulates it and causes it to become an opaque mass, so a similar exposure causes the protoplasm or living material of these organisms to become coagulated and opaque. The conclusion may be drawn that ultra-violet radiation injures or kills living cells by coagulating or rendering insoluble the protoplasm or living material of the cells.

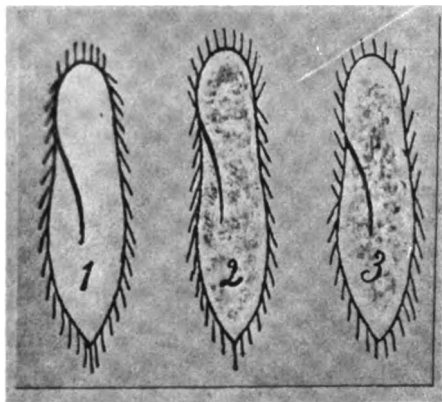


Fig. I. Paramecia. (1) The normal transparent animal. (2) Paramecium killed by ultra-violet radiation. (3) Paramecium killed by heating to 45°C.

Action on proteins. Egg white was poured on a glass plate 6 cm. square and permitted to dry. A piece of cardboard the size of the plate with a circle cut from its center approximately 2 cm. in diameter was fitted over the plate. The preparation was exposed for 30 hours to the radiation from the quartz mercury-vapor burner at a distance of 10 cm. It will be noted that only the central circular area of the egg white was exposed to the radiation since the peripheral portion was covered by the cardboard. At the end of the 30 hours' exposure the cardboard was removed and the plate was photographed.

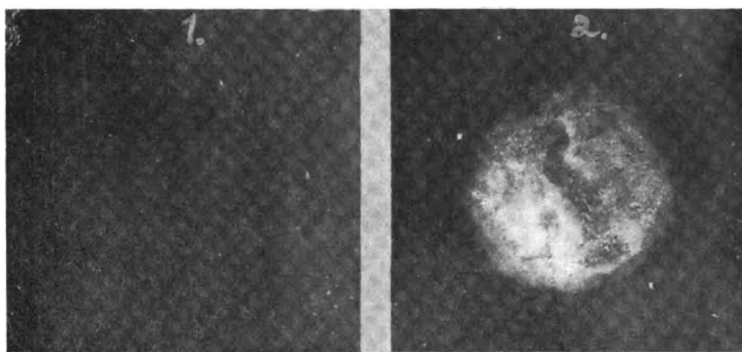


Fig. II. (1) is a photograph of the square of glass covered with egg white, the central area of which had been exposed to ultra-violet radiation. (2) is a photograph of the same square of glass after it had been immersed in a 0.1 per cent calcium chloride solution.

Figure II (1) is the photograph. It may be seen that at this time there was no apparent difference between the exposed central area and the unexposed peripheral area of the layer of egg white. The plate was then immersed in a 0.1 per cent calcium chloride solution. (2) is a photograph of the plate after it had been immersed in the solution for one hour. It may be seen that the calcium chloride precipitated the proteins of the egg white in the circular area where it had been exposed to the radiation, while it did not affect the egg white where it had not been exposed. Calcium nitrate was tried in the same manner and found to have a similar effect in precipitating the protein of the egg white previously exposed to the radiation.

A thin layer of lens material was made by pressing three fresh pig lenses between two quartz plates. The cardboard with a circular area cut from its center was placed over the preparation and this was ex-

posed to the radiation from the quartz mercury burner for 30 hours as the egg white had been. At the end of this time there was no apparent difference between the exposed circular area and the unexposed peripheral area of the lens material, both being transparent. When, however, the preparation was immersed, for about an hour in 0.1 per cent calcium chloride solution the exposed circular area became an opaque mass, while the unexposed peripheral area remained transparent. The conclusion may be drawn that ultra-violet radiation coagulates protein by changing it in such a way that salts such as those of calcium can combine with it to form a coagulum. In this respect it would seem that ultra-violet radiation acts on protein in very much the same way that certain enzymes act on it. It is known that rennin changes the protein in milk, caseinogen, in such a way that calcium salts can combine with it to form a coagulum.

Action of the different wave lengths in the ultra-violet region of the spectrum. An extract of twelve pig lenses was made with 25 cc. of 0.1 per cent calcium chloride solution and filtered through a coarse-grained filter. Five cc. of this fairly clear filtrate were introduced into a circular quartz cell made of two quartz discs having between them a ring of hard rubber 0.8 mm. thick with an inside diameter of 3.8 cm. By means of a small quartz spectrograph the radiation from a quartz mercury-vapor burner operating at 70 volts was focused on the lens extract in the quartz cell. The slit of the spectrograph was 1 mm. wide and the quartz burner was 3 cm. from the slit.

Figure III on following page (1) is a photograph of the spectrum that was focused on the lens extract. (2) is a photograph of the lens extract after the spectrum had been focused on it for 30 hours. The coagulated line of lens protein where the spectral line of wave length $254\ \mu\mu$ was focused, appeared after 50 minutes' exposure; that where the spectral line of wave length $265\ \mu\mu$ was focused appeared after 65 minutes' exposure; that where $280\ \mu\mu$ and $302\ \mu\mu$ were focused appeared after 120 minutes' exposure. The remaining lines of coagulated lens extract appeared after 200 minutes' exposure. (3) is a photograph of the spectrum made on a photographic plate with half the slit of the spectrograph covered with the cornea of a rabbit. It may be seen that the cornea transmits wave lengths as short as $297\ \mu\mu$ and $302\ \mu\mu$ and in (2) it may be seen that these wave lengths are effective in changing the protein of the lens so that calcium salts can combine with it to form a coagulum. (4) is a photograph of the spectrum made through a layer of the lens extract 1 mm. thick. It may be seen that the extract absorbs all wave

lengths shorter than $313\ \mu\mu$ and in (2) it may be seen that all of these absorbed short wave lengths are effective in coagulating the protein of the lens extracted with 0.1 per cent calcium chloride solution. (5) is a photograph of the spectrum through a layer of aqueous humor 1 mm. thick.

In another experiment egg white was introduced into the same quartz cell that had been used with the lens extract. (8) is a photograph of the spectrum of the quartz mercury-vapor burner. (7) is a photograph of the region of the quartz cell containing the egg white where the spectrum had been focused for 30 hours. (6) is a photo-

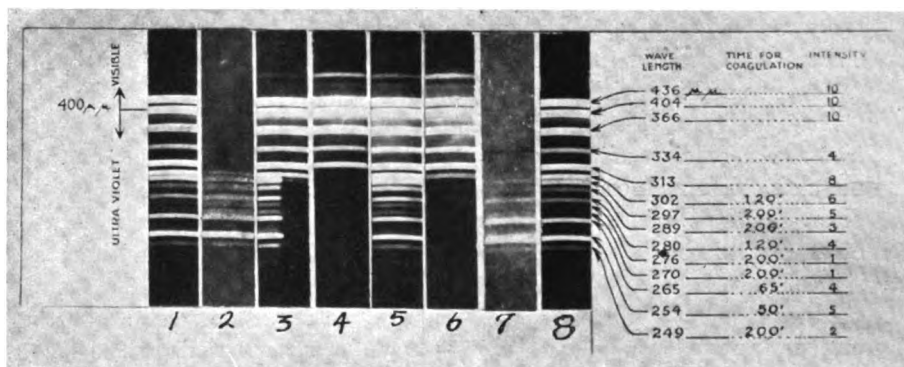


Fig. III. Photograph of spectra of small quartz mercury-vapor burner. (1) made on photographic plate; (2) made on lens protein; (3) made on photographic plate, one-half of the slit of the spectrograph being covered with the cornea of a rabbit; (4) through layer of lens portein 1 mm. thick; (5) through aqueous humor 1 mm. thick; (8) photograph of spectrum made on photographic plate; (7) made on egg white; (6) made on photographic plate through egg white 1 mm. thick.

graph of the spectrum through a layer of egg white 1 mm. thick. It may be seen that the egg white absorbs wave lengths shorter than $302\ \mu\mu$ and that all these absorbed wave lengths are effective in coagulating the proteins of the egg white. The periods of time required for the formation of the lines of coagulated egg white where the spectral lines were focused are indicated in figure III under "time for coagulation." It may be seen that the time required for the coagulation of egg white by the different spectral lines is the same as that required for the coagulation of the modified lens protein by the corresponding spectral lines.

The production of anterior eye trouble in living animals by means of ultra-violet radiation. One batch of six frogs was kept living partially immersed in 0.2 per cent sodium silicate, another batch in 0.8 per cent calcium chloride, another in 1 per cent dextrose, another in tap water for 15 days. At the end of this time one eye of each frog was exposed one hour each day for five successive days to the radiation from a quartz mercury burner operating at 140 volts, 3.3 amperes and 2400 cp. at a distance of 20 cm. Photographs of the frog were made 15 days after the exposures. Figure IV, frog (1) had been living partially immersed in tap water previous to the exposures. Frog (2) had been living partially immersed in 0.2 per cent solution of sodium silicate previous to the exposures. It may be seen that the eyelid of the frog living in the solution of sodium silicate had been converted into an opaque mass, while that of the frog living in tap water was very little injured. The solution of calcium chloride had the same effect as the solution of silicate. The dextrose was effective but not so much so as either of the other solutions. The conclusion may be drawn that salts such as are found to be greatly increased in human cataractous lenses increase the effectiveness of ultra-violet radiation in producing anterior eye trouble.

It is a common experience that the skin sunburns more easily and quickly when it is wet than when it is dry. It is probable that if the skin is dry when it is exposed to sunlight, the ultra-violet radiation in the sunlight changes the protein of the cells of the skin in such a way that salts such as those of calcium in the lymph bathing the cells can combine with it to form a coagulum. If the skin is wet with ordinary fresh or salt water the salts in the water facilitate the process by combining with the proteins of the cells modified by the ultra-violet radiation.

The production of cataract in living animals by means of ultra-violet

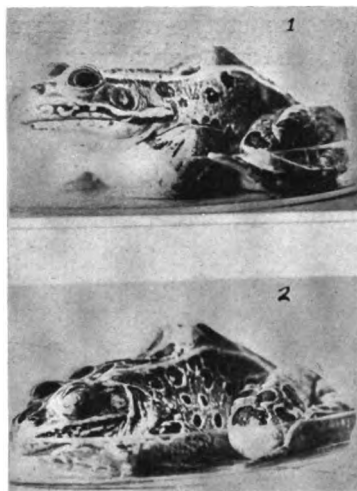


Fig. IV. Frog (1) living in tap water and exposed to ultra-violet radiation for 5 hours. Frog (2) living in 0.2 per cent sodium silicate and exposed to ultra-violet radiation for 5 hours.

radiation. An attempt was made to increase in the fluids of the body of living animals and hence in the eye media the salts found to be greatly increased in human cataractous lenses with the hope that on exposure of the eyes of these animals to ultra-violet radiation cataract would develop. Many observers have demonstrated that it is impossible to produce an opacity of the lens or cataract in the normal living animal by exposure of its eyes to ultra-violet radiation. Moreover, it has been shown that it is not possible to produce an opacity even in excised pig lenses by exposing these directly to the radiation from a quartz mercury-vapor burner for very long periods of time. Fish were chosen for the experiments to be described because they could be kept alive in the solutions of the salts desired. One batch of gold fish was kept in 0.8 per cent calcium chloride solution, another in 0.8 per cent calcium nitrate solution, another in 1 per cent dextrose, another in 0.1 per cent sodium silicate for ten days. At the end of this time each fish in its turn was introduced into a small box with a quartz window in one side. In practice four of these boxes were used so that one eye of each of the four fish was exposed simultaneously. The boxes containing the fish were adjusted so that the quartz windows were 15 cm. from the quartz mercury-vapor burner operating at 140 volts, 3.3 amperes, and 2400 cp. In this manner one eye of each fish was exposed to the radiation. Each exposure was of 6 hours' duration. After the exposure, the batches of fish were replaced in the solutions from which they had been removed. For comparison the eyes of fish living in tap water were exposed in the same manner and for a similar length of time as those living in the salt solutions. As a rule a slight opacity in the cornea of the eye exposed appeared about 15 hours after the first exposure. It was assumed that this opacity did not develop during the exposure because there was not sufficient salt present in the cells of the cornea to combine with the protein modified by the radiation to form a coagulum. However, at the end of 12 or 15 hours a sufficient quantity of salt had collected, owing to the diffusion of the salt from the blood stream into the cornea, and had combined with the modified protein to precipitate it and hence an opacity of the cornea was produced. This assumption would seem to explain the so-called "latent period," i.e., the time elapsing between the exposure of one's eye to ultra-violet radiation and the time when the painful effect is felt. In most of the fish a slight clouding appeared about 2 days after the first exposure in the lens of the eye exposed.

Ten days after the first exposure the eyes of the fish that had been

used were exposed again for another 6 hour period. At the time of this second exposure as a rule an opacity of the cornea and of the lens of the fish living in the salt solutions had increased while the opacity of the cornea of the fish living in tap water had practically cleared up. Several hours after the second exposure as a rule the opacity of the lens and of the cornea of the fish living in the salt solutions became more marked. An opacity of the cornea of the fish living in tap water developed also but this was slight and cleared up in a few days while that of the fish living in the salt solutions increased.

After the second exposure no prescribed rule as to time for the third exposure can be laid down. In order to clear up the opacity of the cornea of the fish in the different salt solutions it was necessary to transfer them to tap water. As a rule the opacity of the cornea cleared up

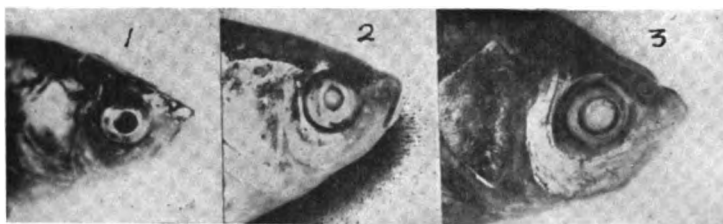


Fig. V. Fish (1) living in tap water and exposed to ultra-violet radiation for 12 hours. Fish (2) living in 0.1 per cent sodium silicate and exposed to ultra-violet radiation for 12 hours. Fish (3) living in 0.1 sodium silicate and exposed to ultra-violet radiation for 24 hours.

in a few days while the lens remained opaque. By nursing, by exposing to ultra-violet radiation, by transferring back and forth from salt solution to tap water it was possible to obtain fish in the condition indicated in figure V. Fish (1) had been living in tap water for 30 days and had been exposed to ultra-violet radiation for two 6 hour periods or 12 hours. Fish (2) had been living in 0.1 per cent sodium silicate for 28 days and had been exposed to ultra-violet radiation for two 6 hour periods or 12 hours. Fish (3) had been living in 0.1 per cent sodium silicate for 42 days and had been exposed to ultra-violet radiation for four 6 hour periods or 24 hours.

It may be seen that the lens of fish (3) living in the silicate solution and exposed to ultra-violet radiation for 24 hours had become perfectly opaque; that of fish (2) living in the same solution but exposed to ultra-violet radiation for 12 hours had become partially opaque while the

lens of fish (1) living in tap water and exposed to ultra-violet radiation for 12 hours was practically clear.

In figure VI are shown the photographs of the spectrum of the small quartz mercury-vapor burner operating at 70 volts and that of a large quartz mercury-vapor burner operating at 140 volts. Photographs showing the effects produced by focusing these spectra on egg white and on lens protein are also given in order to show that the most effective region of the two spectra in precipitating protein are different. (1) is the photograph of the spectrum of the small quartz mercury-vapor burner made on a photographic plate, (9) is a photograph of the spec-

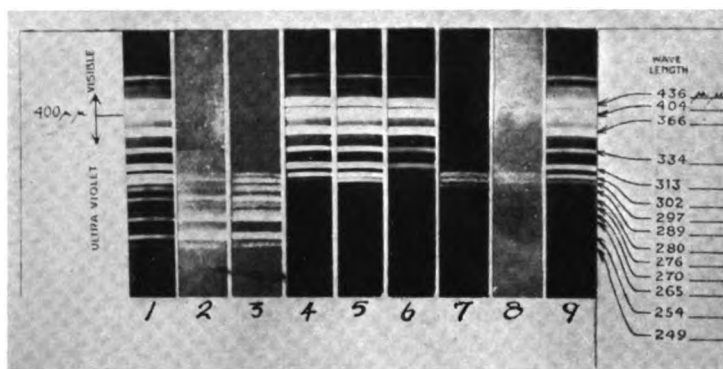


Fig. VI. Photograph of spectra of the small quartz mercury-vapor burner and of the large quartz mercury-vapor burner. (1) That of the small burner; (9) that of the large burner, made on a photographic plate; (2) that of the small burner; (8) that of the large burner, made on egg white; (3) that of the small burner; (7) that of the large burner, made on lens protein extracted with 0.1 per cent calcium chloride solution; (4) that of the large burner made through cornea of the rabbit; (5) made through glass 1 mm. thick; (6) through glass 5 mm. thick.

trum of the large quartz mercury-vapor burner made on a photographic plate. By comparing these two spectrograms it may be seen that the spectral lines in the extreme ultra-violet region of the spectrum are much more intense in (1) than they are in (9). (2) is a photograph of the cell containing egg white on which the spectrum of the small burner had been focused for 30 hours. (8) is a photograph of the quartz cell containing egg white on which the spectrum of the large burner had been focused for the same length of time. By comparing (2) and (8) it may be seen that the egg white was coagulated in (2) where the spectral lines of wave lengths $302\ \mu$ to $249\ \mu$ were focused, while in

(8) the egg white was coagulated only where the spectral lines of wave lengths $302\ \mu\mu$ and $297\ \mu\mu$ were focused. (3) is a photograph of the quartz cell containing lens protein extracted with 0.1 per cent calcium chloride solution on which the spectrum of the small burner had been focused for 30 hours, (7) is a photograph of the quartz cell containing the same kind of extract on which the spectrum of the large burner had been focused for 30 hours. By comparing (3) with (2) and (7) with (8) it may be seen that the same region of the spectrum of the small quartz mercury-vapor burner effective in coagulating the proteins of egg white is also effective in coagulating the lens protein extracted with 0.1 per cent calcium chloride solution. The same statement may be made regarding the effective region of the large quartz mercury-vapor burner in (7) and (8). (4) is a photograph of the spectrum of the large quartz mercury-vapor burner made through the cornea of the rabbit, (5) made through glass 1 mm. thick, (6) through glass 5 mm. thick. It may be seen from (4) that the cornea transmits wave lengths as short as $297\ \mu\mu$ and $302\ \mu\mu$ and from (7) that these are the wave lengths of the spectrum of the large quartz mercury-vapor burner most effective in bringing about the coagulation of the protein of the lens. The large quartz mercury-vapor burner was the one used in the experiments described in which cataract was produced in fish living in salt solutions.

Many observers have attributed their failure to produce cataract in living animals to the protective action of the cornea. I think it will be clear from the experiments reported in this paper that their failure was not due to the protective action of the cornea but to the fact that there were not present in the lens of the eyes of the animals exposed sufficient quantities of salts such as those of calcium to combine with the protein of the lens modified by the ultra-violet radiation to precipitate it. It has been shown that the quantity of calcium in the normal lens is less than 0.08 per cent of the ash and that the amount in human cataractous lenses is about 15 per cent of the ash.

Cataract is of common occurrence among people living in the tropics. Analyses of human cataractous lenses from India show that they contain large quantities of calcium salts and silicates. No silicates were found in human cataractous lenses from the United States. Silicates may be accounted for in the cataractous lenses from India by the fact that silicious earths form a part of the diet of certain classes in India, while the prevalence of cataract in India may be attributed possibly to the comparatively large amount of ultra-violet radiation in

ordinary tropical light and the silicates present in the eye media. The assumption is made that the ultra-violet radiation modifies the protein of the lens so that the silicates can combine with this modified lens protein to precipitate it and hence form an opacity of the lens or cataract.

Cataract is of comparatively frequent occurrence among glass blowers. The eyes of glass blowers are probably subjected to more of the short wave lengths of the spectrum than are the eyes of people generally. It may be assumed that these short waves keep the protein of the lens modified so that if abnormal amounts of the salts of calcium or silicates, etc., are present these combine with the modified lens protein to precipitate it and hence produce an opacity of the lens. Not all glass blowers develop cataract although their eyes are subjected to the same quantity and quality of radiation from the furnaces. It is assumed that those who do develop cataract have a more or less disturbed condition of nutrition expressing itself in an increase of those substances which can combine with the lens protein, modified by ultra-violet radiation, to precipitate it.

CONCLUSIONS

1. Ultra-violet radiation kills living cells and tissues by changing the protoplasm of the cells in such a way that certain salts can combine with the protoplasm to form an insoluble compound or coagulum. The effective region of the spectrum in changing the living material of the cell or protoplasm lies between $254\ \mu\mu$ and $302\ \mu\mu$. The most effective region is around $254\ \mu\mu$ for the small quartz mercury burner used and around $302\ \mu\mu$ for the large quartz mercury burner used.

2. An opacity of the lens or cataract can be produced in fish living in solutions of those salts found to be greatly increased in human cataractous lenses by exposing the eye of the fish to radiation from a quartz mercury-vapor burner. This cannot be done by exposing the eyes of fish living in tap water containing very small quantities of these salts.

3. Abnormal quantities of the salts of calcium and sodium silicate in the cells of the eyelids and of the cornea increase the effectiveness of ultra-violet radiation in producing anterior eye trouble. Abnormal quantities of calcium salts on the skin also increase the effectiveness of the short wave lengths in sunlight in producing sunburn.

THE EFFECTS UPON THE GASTRIC SECRETION OF ORGAN EXTRACTS

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This publication is the fourth in our series upon the physiology of the ductless glands. The first¹ dealt with the effects upon blood pressure of the different substances which can be isolated, without material alteration of their chemical structure, from an aqueous extract of the thyroid gland. We demonstrated in this article that the "residue," or non-coagulable portion, of the extract was the only portion which produced a fall in blood pressure. The second article² dealt with the blood pressure effects of all the similar substances which can be isolated from other organs. In this we demonstrated that the "residue," or non-coagulable portion, of the aqueous extract of all organs (except the adrenal) is the only portion which contains the depressor principle. The adrenal "residue" contains a pressor substance which shows certain marked differences in its effects in the kymograph tracings from those produced by the 1:1000 solution of commercial adrenalin.

When the dose of "residue" is standardized according to its nitrogen content, the residue of each organ produces a characteristic effect in the kymograph tracings. It is, therefore, reasonable to infer that the depressor substance in these residues is peculiar to and characteristic of the organ from which it is derived. The "basic principle" of these depressor substances, which means some particular portion of its molecule, may be the same but the molecule which contains the depressor principle also contains some other molecule or atom or ion, and this addition to the basic depressor principle constitutes an active principle which can be isolated from every organ and is characteristic of and peculiar to that organ.

¹ Fawcett, Rogers, Rahe and Beebe: This Journal, 1915, xxxvi, 113.

² Fawcett, Rogers, Rahe and Beebe: This Journal, 1915, xxxvii, 453.

The term "active principle" is used to signify a substance which can produce some one or more demonstrable physiological effects. In this sense every organ seems to contain at least one active principle, and this differs from that of every other organ.

The kymograph tracings seemed to prove that this active principle of each organ was to be found only in the non-coagulable, or "residue," portion of its aqueous extract. To confirm these observations we next tested the effects of the different bodies which can be isolated from aqueous extracts of organs, upon the contractions of unstriated muscle fiber. In this third report³ we demonstrated that the "residue" or non-coagulable portion of our extracts and no other portion: (a) induced contractions, (b) that these contractions appeared to be characteristic for each "residue," (c) that the peculiar contractions induced by each residue were paralyzed by the subsequent addition of a few drops of a 1:1000 solution of adrenalin. It therefore seemed reasonable to conclude that the active principle in each residue exerted its effects through the terminal filaments or the end plate or the intermediate substance of the distal extremity of the sympathetic nerves. To confirm and elaborate these observations we next tested the effects of our residues upon the secretory activity of readily accessible organs. This fourth report contains the results as regards the stomach.

It is well known that extracts of many kinds made from many different tissues and organs when administered by hypodermic injections affect the flow of gastric juice and also its pepsin and hydrochloric acid content. A recent article on this subject⁴ signifies the material or substance which stimulates the gastric secretion as "gastrin." This suggests the presence, particularly in the epithelial portions of the digestive tract, of a body which has more or less of a specific hormone action upon the secretions of the stomach. But the increase of gastric secretion can be produced by too many substances to warrant the assumption of any hormone or "gastrin" body which acts only upon the stomach. The stimulant action seems to be of a more general character. Although its exact nature can as yet only be surmised, we suggest, from our experiments, that the equivalent of a gastric hormone or gastric stimulant is to be sought in some substance common to many extracts which affects favorably the nutrition and consequently the activity of the gastric epithelial cells and has some relation to their nerve supply.

³ Fawcett, Rahe, Hackett and Rogers: *This Journal*, 1915, xxxix, 154.

⁴ Keeton and Koch: *This Journal*, 1915, xxxvii, 481.

In our tests of the effects of organ extracts upon the gastric juice, we employed dogs in which had been established the Pawlow isolated stomach pouch and fistula. These animals were placed in a retaining frame to which they quickly became accustomed and remained quiet for two or three hours, or a long enough period to permit accurate estimations of the flow from the gastric pouch of its secretion. The size of this pouch, of course, determines the quantity of the secretion and the results are, therefore, relative in each animal. The materials tested were standardized according to their nitrogen content, as in the previous kymograph and muscle experiments. They were administered with every aseptic precaution in measured doses with a hypodermic needle into the subcutaneous tissue of the back or flanks. In our experiments upon blood pressure⁵ it was ascertained that these organ extracts when thus injected subcutaneously even in enormous doses, showed no vaso-dilating effect. Hence it is not at all probable that any increase of the gastric secretion consequent upon the subcutaneous injection of our organ extracts can be caused by splanchnic dilatation. The dogs received no food for at least twelve hours before the tests, and immediately after their conclusion were not rewarded with food. All psychic stimuli were thus carefully avoided. The normal fasting flow during a period of fifteen to thirty minutes was determined before each test. After every injection there was found to be a "latent" period in the flow of gastric juice, lasting about ten minutes. The increase, if it occurred appeared quite suddenly and continued for from one and a half to three hours. This corresponds fairly closely to the results which followed the administration by mouth of test meals of milk and bread, or meat. The total acidity of the collected secretion was determined by titration against $\frac{N}{10}$ NaOH with phenolphthalein as the indicator. The free acid was determined by titration against dimethyl-amido-azo-benzine. Both results are expressed in terms of HCl.

In the first experiments the materials tested consisted of definite and comparable amounts of the substances which can be isolated from an aqueous extract of the pig thyroid gland. These substances in all probability closely approximate, chemically, those which presumably exist during life. They are the nucleoproteins, and the globulins. The filtrate or "residue" which remains after the removal of these bodies and the coagulable proteins, is evaporated to dryness, and is

⁵ Fawcett, Rogers, Rahe and Beebe: This Journal, 1915, xxxvii, 121.

then divisible into an alcohol soluble and an alcohol insoluble substance. The alcohol soluble part can be still further subdivided by the use of basic lead acetate into fractions which we have called, for the sake of convenience, a "lead precipitate" and a "lead filtrate." This "lead filtrate" seemed to contain in our first series of experiments nearly if not all of the active material in the thyroid and other organs. The following tables, I and II, show the results of these tests upon the same dog. They were repeated upon several other animals with approximately the same findings and apparently confirm the localization of an active principle obtainable from the thyroid gland in the "residue" or non-coagulable portion of its extract, and in the particular subdivision of this residue which we have designated the "lead filtrate" from the alcohol soluble part of the original residue.

This final filtrate and, of course, the preceding alcoholic solution, contains a substance which shows a pronounced stimulating effect upon the flow of gastric juice and upon its content of hydrochloric acid. Tests which are not recorded in the table, also show that this increase in quantity and in hydrochloric acid are accompanied by an increase in peptonizing power. The whole "residue" of the thyroid seemed, however, somewhat more active than any of its active fractions.

After testing the effects upon gastric secretion of the thyroid residue, a similar extract was made from muscle tissue. The effects of the subcutaneous injection of the muscle residue and of Witte's peptone and of the ingestion by mouth of milk and beef heart are recorded in the first three lines of Table II. These are entirely negative.

In this same table there are recorded in detail the effects of the injection of the "residues" of the liver, pancreas and spleen and of the pituitary, parathyroid, thymus, pineal and adrenal glands.

The residues of the pituitary, pineal, adrenal and thymus glands and of muscle tissue, as well as Witte's peptone, are practically inert. The other residues are very active gastric stimulants.

We have repeated the tests of effects of extracts made from the beef pituitary (whole gland), the beef pineal and parathyroid, thymus, liver, pancreas, spleen and adrenal glands, and recorded the results in Table III. As the entire thyroid residue seemed a little more active than its fractions, we employed the entire "residue" or non-coagulable portion of extracts of these other organs and compared the effects with those produced by a solution of the combined nucleo-proteins, globulins and coagulable proteins. Except in the case of the pancreas, the coagulable portion of these extracts were uniformly inactive. At the

TABLE

TESTED PORTION OF AQUEOUS EXTRACTION OF THYROID	AMOUNT INJECTED EXPRESSED IN MG. OR PROTEIN	NORMAL COLLECTION FROM PAWLOW FISTULA						SECRETION COLLECTED AFTER INJECTION DURING PERIODS OF 20 MINUTES											
		First 20 mins.			Second 20 mins.			Third 20 mins.			Fourth 20 mins.								
		Time and amount		Total acid	Free acid	Amt.		Total acid	Free acid	Amt.		Total acid	Free acid	Amt.		Total acid	Free acid		
			per cent per cent			cc.	per cent per cent			cc.	per cent per cent			cc.	per cent per cent			cc.	per cent per cent
Nucleo-proteins.....	30 cc.	25 min.	0.2	0.28	0.1	2	0.2	0.25		1	0.2			0.25					
	600 mgm.	2.5 cc.				2	0.16	1	0.17	2	0.12								
Globulins.....	40 cc.	20 min.	0.1	0.12	0.1	2	0.16	1	0.17	2	0.12								
	600 mgm.	2 cc.				20 cc.	0.25	1	0.1	1	0.1								
Alcohol insol. part of residue.....	20 cc.	20 min.	0.12	0.24	0.08	0.25		0.5		1	0.12								
	600 mgm.	1 cc.				1.5	0.12												
Lead precipitate from alc. sol. part of resi- due.....	50 cc.	25 min.	0.28	0.28	0.28	6	0.52	9	0.6	10	0.64	12	0.48			0.6	0.44		
	600 mgm.	1 cc.				5	0.28	8	0.34	7	0.55	3				0.52			
Residue.....	20 cc.	30 min.																	
Lead filtrate from alc. sol. part of residue...	600 mgm.	1 cc.				5		8		11									
Column number.....		2																	

Compare Column No. 2 with Columns Nos. 5, 8 and 11 to perceive at a glance the effect of the injected material upon the quantity of gastric secretion.

TABLE II

MATERIAL TESTED	AMOUNT INJECTED (GUT) IN MG.M. OF PROTEIN	NORMAL COLLECTION FROM PAWLOW FISTULA			First 20 mins.			Second 20 mins.			Third 20 mins.			Fourth 20 mins.		
		Time and amount	Total acid	Free acid	Amt.	Total acid	Free acid	Amt.	Total acid	Free acid	Amt.	Total acid	Free acid	Amt.	Total acid	Free acid
			per cent.	per cent.	cc.	per cent.	per cent.	cc.	per cent.	per cent.	cc.	per cent.	per cent.	cc.	per cent.	per cent.
Muscle residue.....	20 cc. 600 mgm.	20 min. 1 cc.	0.32		1.25	0.32		1.5	0.34		0.5			1	0.34	
Witte's peptone.....	40 cc. 600 mgm.	20 min. 1.5 cc.	0.2		0.5	0.2		0			Total of 2 and 3, 1 cc.	0.2				
300 cc. milk plus 250 gm. beef heart by mouth.....	20 min. 1 cc.	0.23	0.08		1	0.36		5	0.48	0.32	5	0.48	0.32	4	0.44	0.28
Pituitary residue (en- tire gland).....	25 cc. 600 mgm.	20 min. 0.4 cc.	0.5	0	0.6	0.7	0	0.4	0.7	0	0.24	0.9	0	0.4	0.7	0
Parathyroid residue...	35 cc. 600 mgm.	35 min. 0			4.5	0.56	0.36	10	0.6	0.48	10	0.64	0.48	8	0.64	0.48
Thymus residue.....	20 cc. 600 mgm.	20 min. 1 cc.	0.28	0.08	2.5	0.48	0.28	2.5	0.48	0.28	1	0.44	0.2			
Spleen residue.....	20 cc. 600 mgm.	25 min. 1 cc.	0.44	0.24	9	0.6	0.4	13	0.68	0.48	13	0.72	0.52	10	0.68	0.44
Liver residue.....	20 cc. 600 mgm.	25 min. 1 cc.	0.24	0.12	5.5	0.6	0.4	7	0.6	0.4	5	0.6	0.4			
Adrenal residue.....	15 cc. 600 mgm.	25 min. 1 cc.	0.44	0.28	1	0.4	0.24	0			Total of 2 and 3 1.25 cc.	0.24	0.12			
Pineal residue.....	40 cc. 600 mgm.	25 min. 1 cc.	0.4	0.16	3.5	0.52	0.36	1.5	0.52	0.28	0			0		
Pancreas residue.....	6 cc. 600 mgm.	35 min. 1.25 cc.	0.28	0.08	10	0.6	0.44	12	0.72	0.56	13	0.68	0.52	10	0.6	0.44
Column number.....		2			5			8			11					

Compare Column No. 2 with Columns 5, 8 and 11 to perceive at a glance the effects of the different residues upon the quantity of secretion.

conclusion of the tests the pneumogastric nerves in two of the animals were cut through a transthoracic operation. From one to two inches of the nerves were excised just above the diaphragm, as it was found by experiment that simple section was quickly repaired. Two weeks later, or after the wounds had healed and distal degeneration of the nerves had presumably occurred, the tests with the gastric stimulant residues were repeated. There was little or no change in the stimulant effect.

The results recorded in the third table are portrayed less elaborately than the first. The tabulation represents the amount of normal flow with its free and total acid content from the gastric fistula in a fasting dog for the thirty minutes preceding the test injection, and for the second thirty minutes following the injection. Six dogs were employed. Numbers 5 b and 6 b record the results after resection of the thoracic portions of the pneumogastric nerve. In the next to the last line in the first column of the table there occurs the term "desiccated thyroid residue." This refers to the "residue" part of an extract made from a desiccated commercial thyroid powder. The commercial tablets or powder are crude and often unreliable medicaments. These tests, as well as some previously made upon blood pressure, seem to indicate that dessication of the entire gland may destroy much or all of its potency or active principle.

In these, as in the first experiments, the thyroid "residue," or the non-coagulable portion of its aqueous extract, is proved to contain an active stimulant for both the total quantity and the acid content of the gastric secretion.

The liver "residue," or non-coagulable portion of an aqueous extract of the liver, and similar residues of the parathyroid, the spleen and the pancreas are even more active gastric stimulants. Unlike all the others, the coagulable portion of a pancreatic extract was equally active or more active than the pancreatic residue. We can as yet offer no explanation for the reasons of this peculiarity of the pancreas. The pituitary residue and the adrenal residue seemed to inhibit the gastric secretion. The thymus residue in some dogs was a mild stimulant, in others negative. The pineal residue was inactive.

We have been particularly interested in the effects of the thyroid residue and have observed that it not only stimulates gastric secretion but also gastric motility. After opening the abdomen of an animal and observing the contractions of the stomach, we have then injected subcutaneously the thyroid residue. Shortly afterwards there

TABLE III

MATERIALS TESTED	No. 1 PAWLOW STOMACH			No. 2 PAWLOW STOMACH			No. 3 PAWLOW STOMACH			No. 4 PAWLOW STOMACH			No. 5-a PAWLOW STOMACH			No. 5-b PAWLOW STOMACH X NERVE CUT			No. 6-a PAWLOW STOMACH			No. 6-b PAWLOW STOMACH X STOMACH			
	Flow min.	Free HCl per cc.	Total acid per cc.	Flow min.	Free HCl per cc.	Total acid per cc.	Flow min.	Free HCl per cc.	Total acid per cc.	Flow min.	Free HCl per cc.	Total acid per cc.	Flow min.	Free HCl per cc.	Total acid per cc.	Flow min.	Free HCl per cc.	Total acid per cc.	Flow min.	Free HCl per cc.	Total acid per cc.	Flow min.	Free HCl per cc.	Total acid per cc.	
Normal flow.....	0.5	0	0.6	1.5	0.2	0.6	1.8	0	0.9	1.0	0.3	8.0	0.9	1.3	0.3	0.5	1.0	18.0	1.8	2.3	0.3	0	0.5		
Thyroid residue—Subcutane- ously.....	2.0	0.8	1.2	18.0	1.1	1.5	18.0	0.6	1.5	11.0	1.6	16.4	1.0	1.5	1.8	1.0	4.5	28.5	1.9	2.4	8.0	1.7	2.4		
Normal flow.....	0.4	0	0.6				0			1.0	0.7							10.6	0.7	1.0	0.1	0.5	1.0		
Thyroid coagulables.....	0.4	0	0.6				0			1.0	0.4							0.8	1.2	2.0	0.2	0.5	1.5		
Normal flow.....	0.6	0	0.3	1.5	0.3	0.6				1.0	0.7	4.2	1.0	1.2	0.4	0.6	3.7	4.4	0.6	1.6					
Liver residue.....	2.0	0.3	1.5	10.5	1.0	1.5				9.0	1.1	15.0	1.0	1.6	0.7	0.7	4.0	8.4	0.8	2.2					
Normal flow.....	0.3	0	1.5																						
Liver coagulables.....	0.3	1.0	2.5																						
Normal flow.....	0.3	0	1.5	0																					
Parathyroid residue.....	3.0	1.5	1.7	15.0	1.2	1.6																			
Normal flow.....	0.6	0	0.7	0																					
Pancreas residue.....	6.0	0.9	2.2	7.2	0.5	1.3																			
Normal.....	0.4	0	0.5	1.0	0.3	0.8																			
Pituitary residue.....	0.6	0	0.7	0.5	0.1	0.5																			
Normal.....	0.3	0	2.0	1.2	0.2	0.7																			
Pancreas coagulables.....	5.1	1.9	2.2	19.5	1.4	1.8																			
Normal.....	0.3	0	1.5	1.2	0.2	0.7																			
Thymus residue.....	7.5	1.4	2.1	4.0	0.7	1.2																			
Normal.....	0.4	0	0.6																						
Thymus coagulables.....	0.4	0	1.0																						
Normal.....	0.7	0.7	2.0	1.5	0.7	1.1	0.6																		
Adrenal residue.....	0.5	1.0	2.5	1.0	0.3	0.6	0.3																		
Normal.....	0.4	0	1.1	1.5	0.6	1.1																			
Spleen residue.....	5.2	1.0	1.1	19.5	1.3	1.8																			
Normal.....																									
Desiccated thyroid residue.....																									
Normal.....				1.5	0.2	0.7	4.0				0.7														
Food.....				7.5	0.8	1.2	27.0				1.6														

'Res.' or 'Residue' refers to the non-coagulable portion of the aqueous extract of an organ. 'Coag.' or 'coagulables' refers to the substances in solution in the remaining part of the extract.

regularly occur in the stomach very vigorous peristaltic waves. These continue for a much shorter time than the increased secretion, but they regularly follow the injection of even small amounts of the thyroid residue. Sometime in the future we hope to depict this interesting phenomenon graphically. Thus far our attempts have been rather unsatisfactory. The residues show no change in their stimulating effects upon gastric secretion after resection and degeneration of the pneumogastric nerves.

Pawlow has demonstrated the powerful stimulant effect of the tenth cranial nerve upon gastric secretion. It is presumable that all of these stimulant residues act through some peripheral mechanism. From our previous experiments, this can be inferred to be either purely nervous or a combination of the nervous, cellular, and vascular elements.

CONCLUSIONS

1. The thyroid, or only the non-coagulable portion of an aqueous extract of the thyroid, contains a substance which is an active stimulant of both the gastric secretion and the gastric motility.
2. Only the residue portion of an aqueous extract of the parathyroid and thymus glands, and of the spleen and liver, have a similar effect.
3. Both the coagulable and the non-coagulable portions of an aqueous extract of the pancreas are very vigorous stimulants of gastric secretion.
4. The pituitary and adrenal residues inhibit the flow of gastric secretion.
5. All the stimulant residues seem to act upon some peripheral gastric mechanism in which the nervous system is an essential part.
6. The residue, or non-coagulable portion of an aqueous extract of the pituitary, pineal, thyroid, parathyroid, thymus and adrenal glands, and of the spleen and liver, contains all of the material which shows a demonstrable action upon other organs.

Pitt

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THE INFLUENCE OF HEAVY METALS ON THE ISOLATED INTESTINE

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FIRST COMMUNICATION

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The pharmacology of the isolated intestine has been made the subject of a number of investigations within recent years. Kuliabko and Alexandrowitsch (1) were the first to test the action of different drugs by this method. A similar investigation was carried out later by Magnus (2) on the intestine of the cat, while Kress (3) studied the action of the same drugs on the intestine of the dog and of the rabbit. Sembdner's (4) experiments with chloral, Kuno's (5) work with the alcohols, the studies on the action of members of the fatty acid series made by Rona and Neukirch (6), Starkenstein's (7) studies with calcium precipitants and the recent communications of Hanzlik (8) on chelidonin may also be mentioned in this connection. The tests on pilocarpine made by Neukirch (9) mark the first attempt at quantitative pharmacological studies on isolated segments of intestine. This method was also employed by Kuyer and Wijssenbeck (10) for the investigation of the antagonistic action of drugs.

The influence of the heavy metals on the intestine has received very little attention as yet, Siccardi's (11) experiments with lead acetate on the intestine of the rabbit being the only communication on the subject we could find in the literature. The present report aims at presenting some results obtained with zinc, in the form of malate, and nickel, of which the acetate was used, on different parts of the small intestine and colon of the cat and the rabbit.

Studies were also made on the reaction to barium chloride, pilocarpine and atropine after being subjected to the influence of the heavy metals. The method devised by Magnus and carried out in this laboratory as described in a recent publication by us (12) was also used in the present investigation. The zinc, as well as the nickel salt, was dissolved and added to Locke solution which was maintained at a temperature of 37 to 38° C.

The action of zinc. The toxicity of zinc has been established by experiments on lower organisms, as well as on higher animals. According

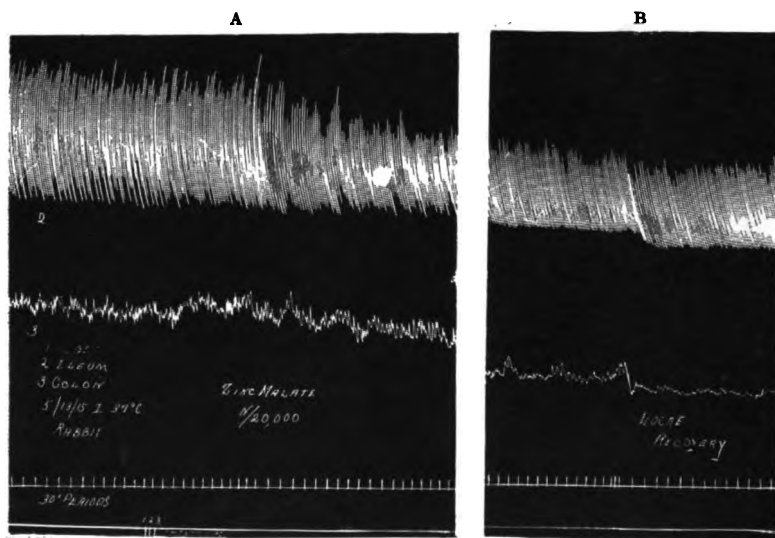


Fig. 1. Rabbit 1789. A, in zinc malate $N/20,000$, 15 minutes. B, in zinc malate at the end of 43 to 60 minutes. Also shows contractility in Locke solution alone after previous treatment.

to Freitag (13) its presence in nutrient solutions in a concentration of 0.02 per cent kills the roots of some phenoragamic plants. More recently Hawkins (14) has obtained similar results in experiments on algae. According to Harnack (15) the administration of zinc salts to higher animals causes symptoms of depression and paralysis of the muscles, respiration and circulation.

Experiments on the isolated intestine of the rabbit. Segments of the small intestine and of the colon manifested signs of decreased activity soon after they were suspended in a dilute solution of zinc salt. Very

low concentrations, $N/20,000$ (fig. 1) and in one case $N/30,000$, caused a well marked depression. The concentration was gradually increased, but a marked difference in the results was first observed when a solution of $N/10,000$ was tried. After a brief period of stimulation involving tonus and rhythmic action, depression set in and continued steadily 15 to 60 minutes; the contractions then remained uniform but much reduced in size (figs. 2, 3). Occasionally decreased frequency of action could also be observed at this stage. The course of events varied somewhat in different parts of the intestine. Rise of

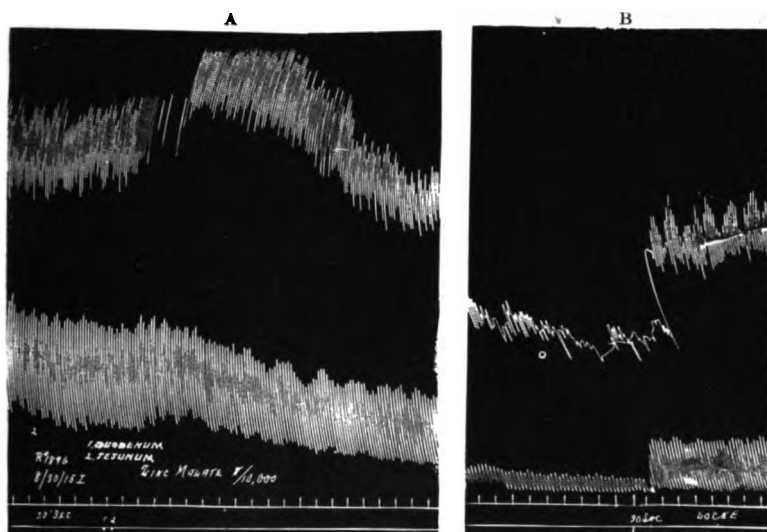


Fig. 2. Rabbit 1896. Zinc malate $N/10,000$. A, after 10 minutes. B, after 35 to 45 minutes and when changed to pure Locke solution.

tonus and irregularity were more frequent in the duodenum; decrease of tonus was sometimes noticed in the colon, but such changes seldom occurred in the jejunum and ileum. When suspended in pure Locke solution again, after thoroughly washing, considerable improvement was observed even when contact with zinc malate lasted 70 minutes. Complete recovery, however, never occurred although the action of zinc was in some cases limited to a period of 45 minutes only. That the tissues were permanently damaged also appeared in experiments, which showed the effect of several treatments with the salt. The preliminary stimulation was absent while depression set in almost imme-

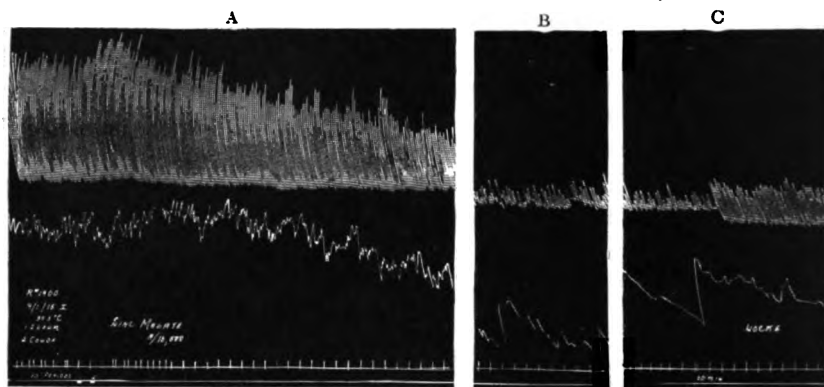


Fig. 3. Rabbit 1900. Zinc malate $N/10,000$. A, shows initial stimulation followed by depression of ileum and colon. B, contractions of the ileum much reduced in size at the end of 46 to 52 minutes in zinc malate; tonus decreased in colon. C, moderate improvement when returned to Locke solution shown, amplitude of rhythmic contractions and tonus of ileum increased when changed to pure Locke solution but did not recover.

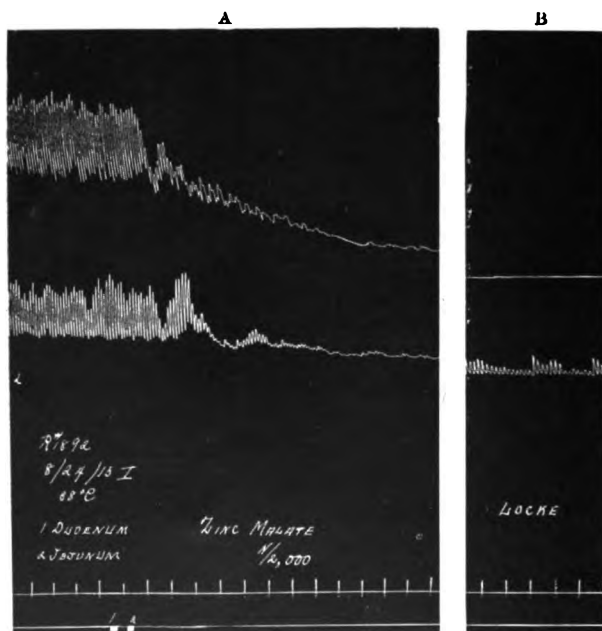


Fig. 4. Rabbit 1892. Duodenum and jejunum. Zinc malate $N/2000$. A, marked depression. B, shows extent of recovery when returned to Locke solution alone. Segments subjected to zinc malate for 30 minutes.

diately after the zinc salt was added, the progressive decrease of amplitude being much faster than in experiments in which the intestine was subjected to the action of zinc for the first time. Contractility was abolished within 28 minutes in the latter case while the same effect was produced in a few minutes after the second treatment with zinc salt. A moderate amount of decrease in the rate of contraction was also observed. When suspended again in pure Locke's solution the improvement noticed was slight in one segment while contractions were absent in another, although it was exposed to the action of zinc salt for a period of about 30 minutes only.

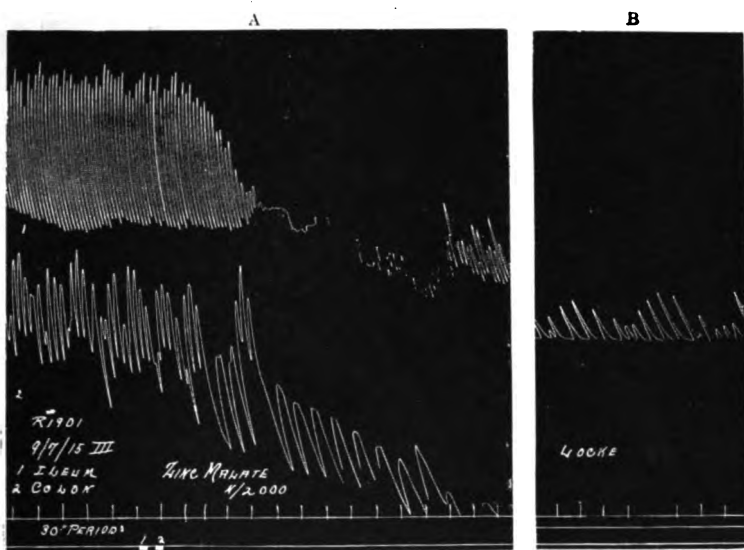


Fig. 5. Rabbit 1901. Ileum and colon. A, effect of zinc malate $N/2000$. B, recovery in Locke solution after subjection to zinc malate for 45 minutes.

The action of $N/5,000$ zinc malate was more marked than in experiments with $N/10,000$, the difference observed being appreciable. The preliminary rise, however, could still be noticed in some instances. Depression of rhythmic contractions and of tonus set in promptly upon the addition of the salt and reached a maximum within 6–20 minutes after the zinc malate was added. The contractions disappeared or became very weak at this time. Irregular action also appeared. Recovery when placed in Locke's solution after exposure to the action of the zinc salt for 40 to 45 minutes was incomplete. Only slight

improvement in contractility was observed in some experiments in the duodenum and jejunum. The contractions in the ileum were pronounced but were less forcible than in the fore period.

The results obtained in experiments with higher concentrations indicate that the activity of zinc malate was considerably greater but the difference was not in proportion to the amount of the salt present in solution. Depression without initial stimulation was observed with

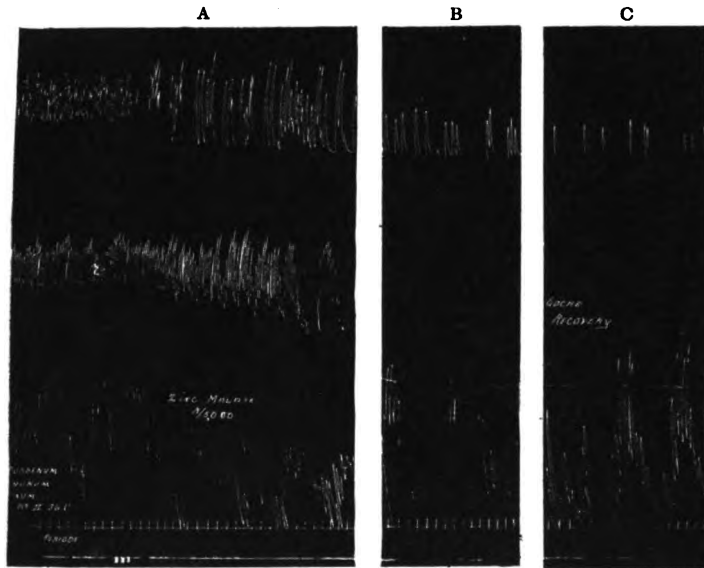


Fig. 6. Cat 370. Zinc malate $N/5000$. Duodenum, jejunum and ileum; upper, middle and lower tracings. A, primary stimulation of duodenum and jejunum with depression of ileum. B, 46 to 54 minutes in zinc malate. Frequency of rhythmic contractions decreased in duodenum, abolished in jejunum. Ileum shows improvement. C, the effect of pure Locke solution. Duodenum. Contractions less frequent than before. Jejunum. No contractions. Ileum shows stimulation.

a solution of $N/2000$ (figs. 4 and 5) after 1.5 to 2 minutes in some experiments; in others this occurred in about 30 seconds after the zinc malate was added. Approximately 4 minutes usually elapsed before contractions ceased in the duodenum and jejunum, but the suppression of activity was delayed considerably in the ileum. Rhythmic contractions, though feeble, persisted 20 to 25 minutes in some experiments after zinc malate was added. Eleven minutes was the shortest period

of activity of the ileum as a result of treatment with $N/2000$ solution. After being in contact with malate 17 to 45 minutes and then placed in Locke's solution, contractility was slight, or absent in the duodenum and jejunum but was distinct and in some cases well marked in the ileum, thus showing once more the greater resistance of this portion of the intestine to zinc malate. When the concentration was increased to $N/1000$ pronounced depression was observed almost immediately, or within half a minute after adding the salt. The contractions ceased in two to five minutes. Depression of tonus was very marked in some sections of the intestine, but was absent in others. When suspended in Locke's solution alone very feeble contractions, or none at all, were observed in the duodenum and jejunum, even when previous treatment with zinc malate was only eight minutes, but the improvement in the ileum was constant. Tests were also carried out with $N/500$ zinc malate. Marked depression of tonus and complete cessation of rhythmic activity set in promptly after the addition of salt.

Experiments on the intestine of the cat. The results obtained show that the response to zinc is less marked in the intestine of these animals than in that of the rabbit. (See figs. 6 and 7). A solution of $N/5,000$ zinc malate produced depression in the duodenum and jejunum in about 25 minutes which proceeded gradually to complete extinction of the contractions about a half hour later. In nearly every case this followed stimulation which was preceded sometimes by initial depression occurring promptly after the addition of the salt. Although the activity of the ileum was also decreased, complete inhibition of contractility was never observed. That this portion of the intestine is more resistant to zinc was also shown by its recovery when it was returned to Locke's solution, whereas neither the duodenum nor the jejunum showed any signs of improvement when subjected to the same treatment.

In a series of experiments with $N/2,000$ zinc malate two types of response could be distinguished. In one, gradual depression set in and continued steadily until all contractility disappeared within 18 to 25 minutes, sometimes within 8 minutes. In the other type, depression set in promptly and contractions disappeared, but returned at the end of 6 to 16 minutes. When placed in Locke's solution, improvement occurred in only one experiment on the ileum. A noticeable difference in the behavior of the intestine was observed when it was treated with stronger solutions. Contractions disappeared promptly in $N/500$ and in 6 to 10 minutes in $N/1,000$ zinc malate. In one experiment,

however, activity continued 28 minutes. A return of contractility was never observed even when Locke's solution was substituted. It may be remarked that the ileum showed greater resistance also in these experiments as it continued its activity in one case 10 minutes in $N/500$ solution and 45 minutes in $N/1,000$, though the strength of

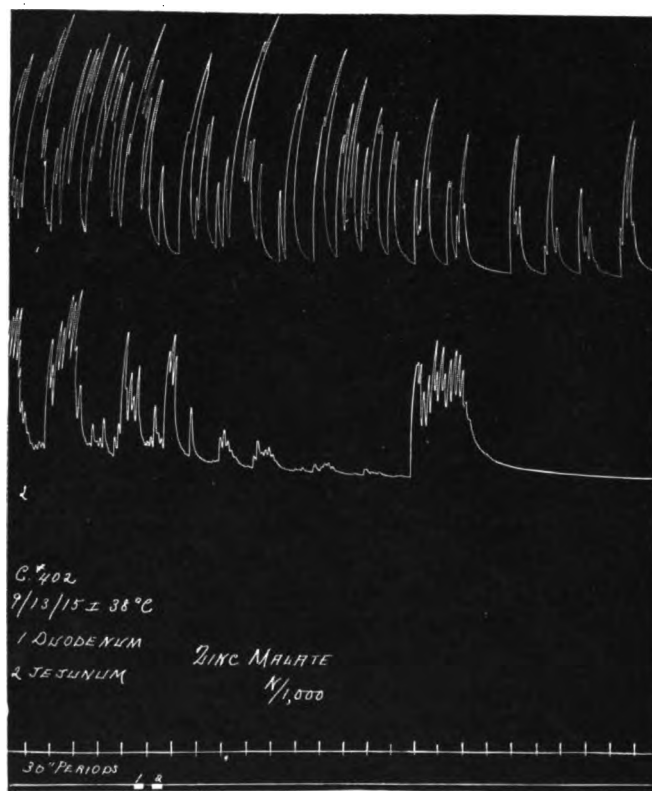


Fig. 7. Cat 402. Note contractions of duodenum after 10 minutes' exposure to $N/1000$ zinc malate.

the contractions was considerably reduced. It might be added that in this case weak but distinct contractions were observed when pure Locke's solution was substituted for one containing zinc salt.

Reaction to pilocarpine, barium and atropine. The tests were carried out on rabbit's intestine in the presence of zinc salt, these substance being added to the solution at various intervals, thus permitting

of the study of the influence of time on the reaction of the intestine. Absence of the normal response to pilocarpine could be observed shortly after the intestine was subjected to the influence of a weak solution of zinc malate. Pilocarpine hydrochloride, 1 : 200,000, produced only moderate stimulation in the jejunum and ileum $4\frac{1}{2}$ minutes after these segments had been subjected to the influence of $N/5,000$ zinc malate. In another experiment the reaction to pilocarpine tested after the intestine had been subjected to $N/5,000$ zinc malate for 22 minutes caused but a slight response. A reaction to pilocarpine could still be obtained when higher concentrations, $N/2,000$ and $N/1,000$ of zinc malate were used; fifteen minutes after the segments of the jejunum and ileum had been suspended in $N/2,000$ zinc malate and contractions had disappeared, a slight reaction to pilocarpine was produced. The same concentration of the alkaloid was still effective in the presence of $N/1,000$ zinc malate after 15 minutes. A slight response was observed in the ileum in another experiment in which the test was made after 21 minutes, but no reaction to pilocarpine was observed in a third experiment with $N/1,000$ zinc malate. It might be added that the intestinal segments were in a state of relaxation or paralysis in all experiments with $N/2,000$ and $N/1,000$ and at the time pilocarpine was tested. Only the jejunum was in this condition after 22 minutes in $N/5,000$ zinc malate. Experiments in which pilocarpine preceded treatment with zinc salt were also performed. The stimulation produced by the alkaloid was promptly antagonized by a solution of $N/5,000$ of the salt, and the activity of the intestine gradually decreased as in the experiments with zinc alone.

The reaction of the zinc treated intestine to barium chloride was preserved considerably longer and was much more pronounced than the response to pilocarpine (see fig. 8). Some time after spontaneous contractions disappeared and no reaction to pilocarpine could be obtained, very marked stimulation with barium chloride could be induced. Thus in two experiments with $N/5,000$ zinc malate, a response was obtained in one case 17 minutes after all contractions ceased; in another experiment a reaction was observed in a paralyzed segment of the jejunum which had been acted upon by zinc for 42 minutes. When treated with solutions of $N/2,000$ and $N/1,000$ zinc malate similar results were obtained. The addition of barium chloride after 30 minutes exposure of the intestine to zinc salt was followed by the appearance of contractions. Spontaneous movements before this test was made were absent. Attempts to obtain a reaction with pilocarpine were

unsuccessful. The reaction to atropine in the presence of zinc was likewise tested in three experiments. It may be recalled that Kress has shown that small amounts of atropine stimulate intestinal contractility. No effect was obtained in two experiments, and well marked depression was produced in the third, $N/5,000$ and $N/2,000$ zinc malate being used in these tests.

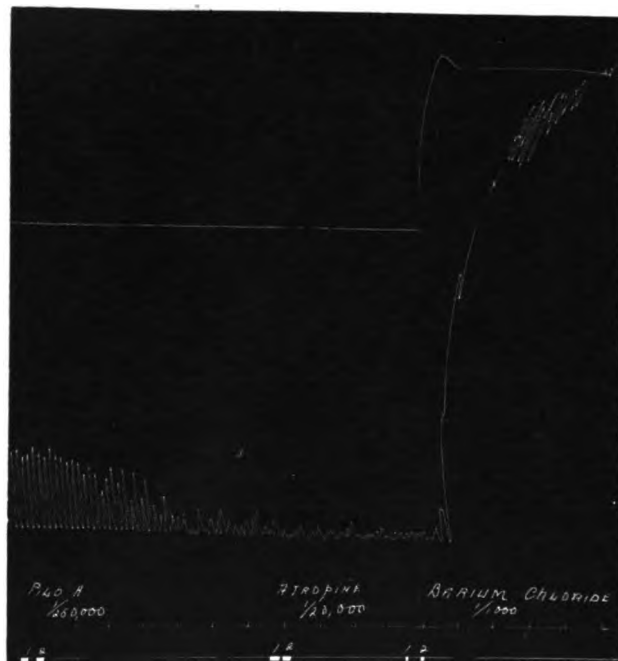


Fig. 8. Rabbit 1905. In $N/2000$ zinc malate four minutes. Upper tracing, jejunum; lower, ileum. Neither pilocarpine hydrochloride 1 : 250,000 nor atropine sulphate had any effect but barium chloride caused prompt and very pronounced contractions.

The following two protocols are typical of the experiments with the reaction to pilocarpine, barium and atropin:

R. 1904. One minute after jejunum and ileum were suspended in $N/5,000$ solution of zinc malate marked depression and irregularity were observed, the contractions being very weak in the former. Twenty two minutes later 1 : 200,000 pilocarpine hydrochloride produced slight stimulation in the jejunum. Very weak contractions appeared at inter-

vals of about a minute or more. The effect on the ileum was greater, though less than on the normal intestine. Eight minutes later the same amount of pilocarpine was added again, the reaction was slightly greater in the jejunum but was weaker in the ileum than in the previous test. At the end of 50 minutes exposure to zinc malate the jejunum ceased to contract but the ileum was still active. Barium chloride 1/1,000 produced a well marked reaction which was much greater in the ileum than in the jejunum.

R. 1905. The effect of pilocarpine in the proportion of 1 : 250,000 was tested four minutes after the segments of the jejunum and ileum had been exposed to the action of N/2,000 zinc malate. The contractions of the ileum which were fairly strong before the alkaloid was added to the Locke's solution steadily decreased until they became very weak and irregular thirty minutes later, while the jejunum remained inactive as before the pilocarpine was introduced into the solution. Barium chloride which was added five minutes after pilocarpine produced a powerful contraction in the ileum, the tonus remaining very high for a considerable length of time, rhythmic contractions also appeared. The jejunum likewise shared in this stimulation but to a much smaller extent. Neither segment was influenced by the previous addition of atropine sulphate.

The action of nickel. The effects of nickel on different animals has been studied by Gehrkens (17), Stuart (18) and Bulatow (19). Severe symptoms and death were observed after the subcutaneous and intravenous administration of small doses of nickel salts. According to Stuart and to Bulatow a fall of blood pressure is also caused when the salt is injected intravenously. That it is toxic to lower organisms appears from the observations of Hawkins (20) who studied the behavior of fungus spores toward nickel nitrate.

Experiments on the intestine of the cat. Various concentrations were employed in these tests. Solutions of N/50,000 and N/20,000 failed to produce any demonstrable change although allowed to remain in contact with the tissues for a considerable period of time. A distinct after effect could be observed, however, in a few cases in which total suppression of rhythmic contractions occurred when the solution of nickel acetate was replaced by pure Locke solution. Definite results, though not very pronounced, were obtained with a solution of N/10,000. Depression and sometimes complete abolition of rhythmic action with decreased tonus were the first manifestations of a reaction to the metal and lasted 25 to 35 seconds. This was followed, however, by a period

of progressive improvement ending in recovery in the jejunum while in the ileum tonus as well as rhythmic contractions became even greater than in the fore period (figs. 9 and 10). In neither case was the sub-

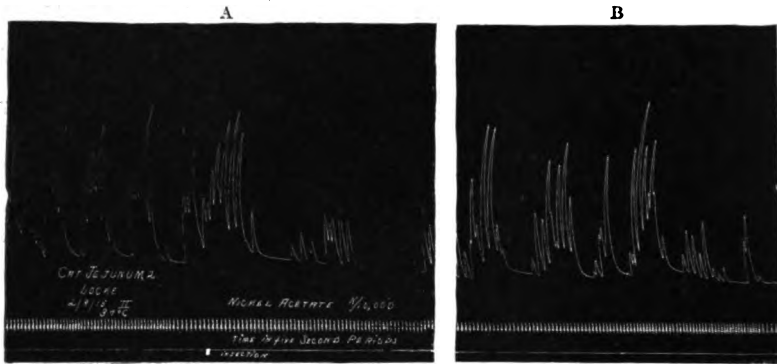


Fig. 9.

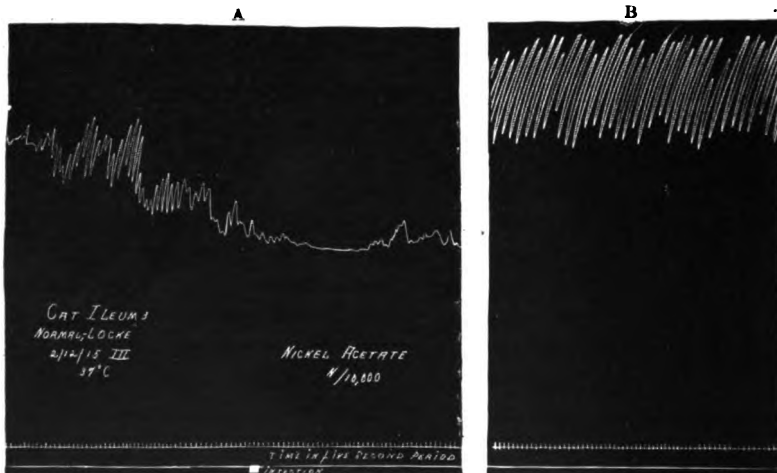


Fig. 10.

Fig. 9. Cat 328. A, primary depression. B, contractions after 25 minutes in nickel acetate.

Fig. 10. Cat 331. A, primary depression. B, stimulation after one hour in $N/10,000$ nickel acetate.

stitution of pure Locke solution followed by any noteworthy changes. A few experiments were also carried out with $N/5,000$ nickel acetate. Depression of tonus with suppression of contractions were present as

in experiments with $N/10,000$ nickel acetate, but recovery was delayed longer than in the latter. Prompt and total inhibition of muscular contraction with decrease of tonus was more frequent and the effect more lasting with higher concentrations. Segments of the jejunum and ileum which were treated with $N/2,000$ nickel acetate ceased their activity almost immediately. They were under observation 12 to 18 minutes, but no change could be noticed. The effect on the colon was less marked. Complete abolition of contractions was exceptional. In most experiments moderate depression was the only effect produced in the colon. This also occurred with a solution of $N/1,000$ shown in Cat 386 (fig. 11) in which pronounced depression of the colon was soon

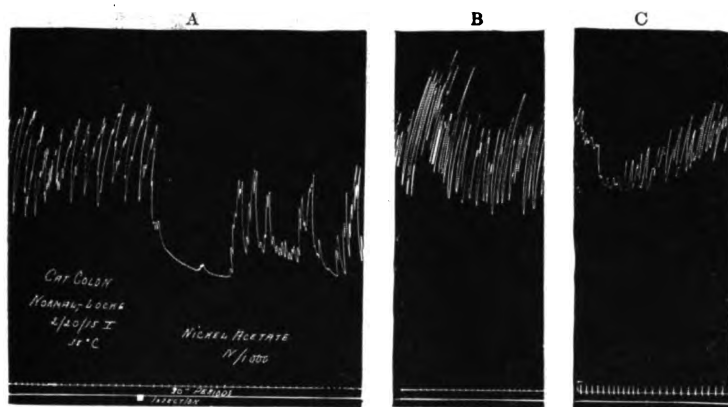


Fig. 11. Cat 386. Colon in nickel acetate $N/1000$. A, depression of tonus and rhythmic contractions. B, condition of intestine $1\frac{1}{2}$ hours after adding nickel acetate. C, condition in pure Locke solution.

followed by improvement ending in recovery while still in nickel acetate. In another experiment on the colon with the same concentration, the primary depression was followed by recovery and stimulation while still in nickel acetate. The injury caused by nickel acetate is not permanent, however, as shown in experiments with the higher concentrations. It was noticed that when pure Locke solution was substituted for $N/2,000$, $N/1,000$ and $N/500$ nickel acetate, improvement and sometimes complete recovery took place although it had been acted upon by the metal for periods of time varying between 25 and 54 minutes.

The action of nickel on the intestine of the rabbit. Disturbance of muscular action in the intestine of the rabbit was observed even when

low concentrations of nickel acetate were employed. A solution of $N/10,000$ nickel acetate already produced marked effects causing at first depression then stimulation of tonus accompanied by a decrease and irregularity of amplitude of the rhythmic contractions. It occurred almost immediately after the metal was added to Locke solution and lasted 2 to 6 minutes. This was succeeded by gradual increase in the force of contractions, the amplitude becoming appreciably greater than in the fore period, but the rate remained unchanged. Within 12

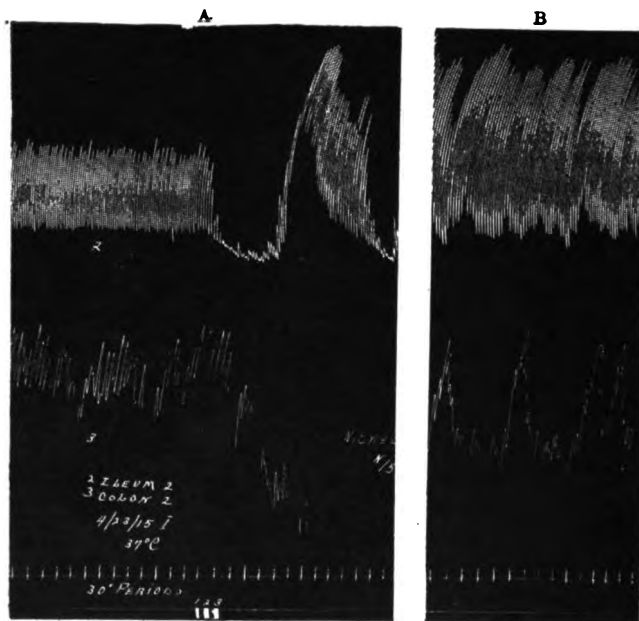


Fig. 12. Rabbit 1803. Ileum and colon. A, segments subjected to nickel acetate $N/5000$. B, 45 minutes after addition of nickel acetate.

to 18 minutes after the test with the metal was begun the progressive rise of the amplitude ceased and remained uniform to the end of the experiment. Although the results obtained were practically the same in most cases, a few exceptions were noted. The addition of nickel acetate was followed in some tests on the jejunum by gradual depression without recovery. The action of $N/5,000$ nickel acetate differed but little from that caused by a solution of $N/10,000$. Depression of rhythmic contractions was followed by gradual recovery in the presence of the salt (fig. 12). In some experiments dis-

tinct stimulation was noticed, the contractions becoming stronger than in the preliminary period. Temporary disturbance of tonus was likewise observed. Most of the experiments were carried out on the jejunum, but tests were also performed on the duodenum and ileum. When pure Locke solution was substituted for one containing nickel acetate, marked decrease of rhythmic contractility was observed after a solution of $N/10,000$ as well as after $N/5,000$. The depressive action of more concentrated solutions was more pronounced. $N/2,000$ nickel acetate produced in the various sections of the small intestine and in the colon a marked decrease of the size of the rhythmic contractions, frequency not being affected. Marked decrease of tonus was also noticed. Gradual improvement while still in the salt solution took place, but the maximum strength obtained was below that in the fore period. In one case only was stimulation with considerable increase of amplitude observed. A marked fall of tonus occurred in the duodenum and lasted 3 to 5 minutes but recovered gradually.

A solution of $N/1,000$ nickel acetate promptly abolished rhythmic contractions, tonus also being markedly depressed at the same time in nearly all experiments. Improvement was observed in all experiments with this concentration but it was delayed considerably. Contractions began to return in some cases after 7 to 9 minutes while sometimes it was delayed 22 minutes. It may be remarked in this connection that similar effects although much less frequent were observed with much more concentrated solutions. Segments of the jejunum and ileum suspended in $N/500$ nickel acetate ceased to contract promptly upon the addition of the salt. During a period of observation lasting 17 minutes no change was observed in the jejunum, but contractions returned in the ileum and became appreciable in 3 minutes.

Reaction to pilocarpine and barium. That the intestine preserved its irritability after nickel acetate was also shown by tests with pilocarpine and barium chloride. Although contractions were abolished when the concentrations used were sufficiently high the addition of pilocarpine promptly restored muscular activity. Powerful contractions appeared in segments of the intestine previously made inactive by a solution of $N/1,000$ nickel acetate. The effect also showed a tendency to continue over a considerable period of time, no abatement of the stimulating action of pilocarpine could be noticed during periods of observation of 16 and 28 minutes. The effect varied with $N/500$ nickel acetate, the reaction to pilocarpine being very pro-

nounced in some experiments, while a weak reaction only was observed in another experiment; tests in both cases were made on jejunum and ileum. Failure to react to pilocarpine was first observed in some experiments with $N/200$, while no reaction could be noticed after $N/100$ was used. Barium chloride when added in sufficient amount, however, produced a distinct response. Tonus and rhythmic contractions reappeared. The stimulating effect of barium was also noticed in a number of experiments in which no reaction to pilocarpine could be obtained. Thus in one experiment on the jejunum and ileum a rise of tonus only occurred in the jejunum, and rhythmic contractions of moderate strength were observed in the ileum when barium chloride $1/500$ was added 25 minutes after the intestine had been suspended in $N/100$ nickel acetate. Pilocarpine hydrochloride tried 10 minutes before barium had no effect. Similar results were obtained with eserine and barium chloride on the intestine of the cat which had been subjected to the action of $N/1,000$ nickel acetate. Barium chloride $1:500$ which was added 12 minutes after nickel acetate, stimulated the duodenum, jejunum and ileum so that the contractions became considerably stronger than in the fore period while eserine in the proportion of $1:100,000$ had no effect.

DISCUSSION

Although depression may be produced by the more concentrated solutions of either metal, a marked difference in their effect followed exposure of the intestine to dilute concentrations of zinc or nickel. In experiments on the rabbit's intestine, a solution of $N/10,000$ zinc malate caused depression of tonus and decrease of amplitude within one-half to 3 minutes. At the end of three-quarters to one hour, and sometimes after 15 minutes only, rhythmic contractions were reduced to a small portion of their original size. The same concentration of nickel acetate sometimes caused primary depression for a brief period but was usually followed by recovery and stimulation. This was also observed in experiments with a solution of $N/5,000$ nickel acetate. That the toxicity of zinc is greater than that of nickel was further indicated in experiments with higher concentrations. Exposure to a solution of $N/500$ nickel acetate was followed by a return of contractions when the intestinal segments were transferred to pure Locke's solution. Sometimes rhythmic contractions reappeared while the intestine was still in contact with the salt. That the depressing effect was more marked with solutions of zinc malate than with nickel was also shown.

when weak contractions only, much reduced in frequency, were observed as the ileum was transferred from $N/2,000$ zinc malate in which it remained 20 minutes, to pure Locke's solution. In another experiment however the jejunum made almost a complete recovery in pure Locke's solution in which it remained 8 minutes. The difference in the reaction of the intestine of the cat to zinc and nickel was not so clearly defined as in the case of the rabbit but the tendency to greater depression as a result of treatment with zinc was shown as recovery in Locke's solution occurred after being treated with $N/2,000$, $N/1,000$

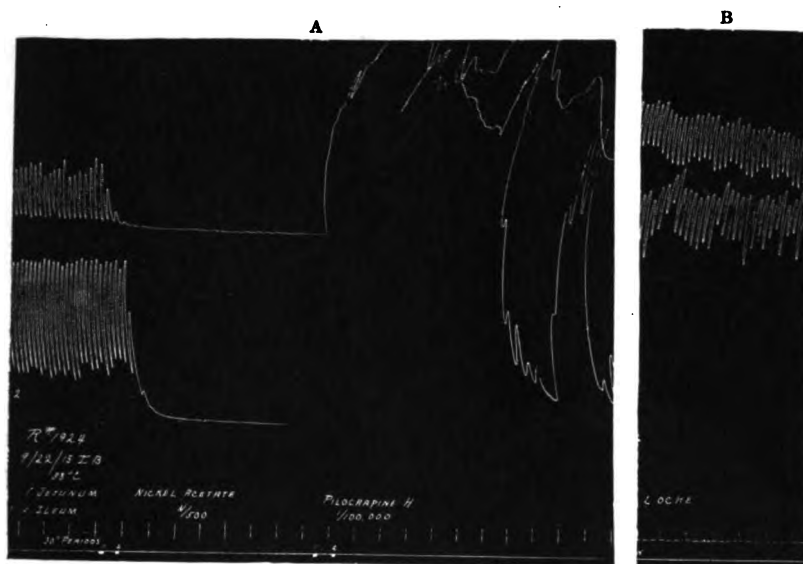


Fig. 13. Rabbit 1924. 1 B. A, shows stimulating effect of pilocarpine after four minutes in $N/500$ nickel acetate. B, contractions in Locke solution after 33 minutes in nickel acetate.

and $N/500$ nickel acetate, whereas the same concentrations of zinc malate had rendered the cat's intestine incapable of recovery in pure Locke's solution. Previous observations on the reactions of the intestine of different animals to various substances were made by Kuyer and Wijzenbeek (22) who have likewise shown that the intestine of the cat and rabbit differ in their behavior towards tyramin as this substance depressed the former and stimulated the latter. According to Magnus (23) and Kress (24), marked differences were also observed in the effects of physostigmin and nicotine on the isolated intestine of various animals.

That zinc is more toxic than nickel was also shown very distinctly by the reaction to pilocarpine and barium chloride. Thus the effect of pilocarpine was slight after 25 minutes exposure to $N/5,000$ zinc malate and 15 minutes exposure to $N/2,000$. When subjected to the action of $N/1,000$ zinc malate for 15 minutes little stimulation with pilocarpine could be obtained. This condition was first observed in experiments with $N/200$ nickel acetate, but stimulation with pilocarpine was pronounced when concentrations of $N/1,000$ and $N/500$

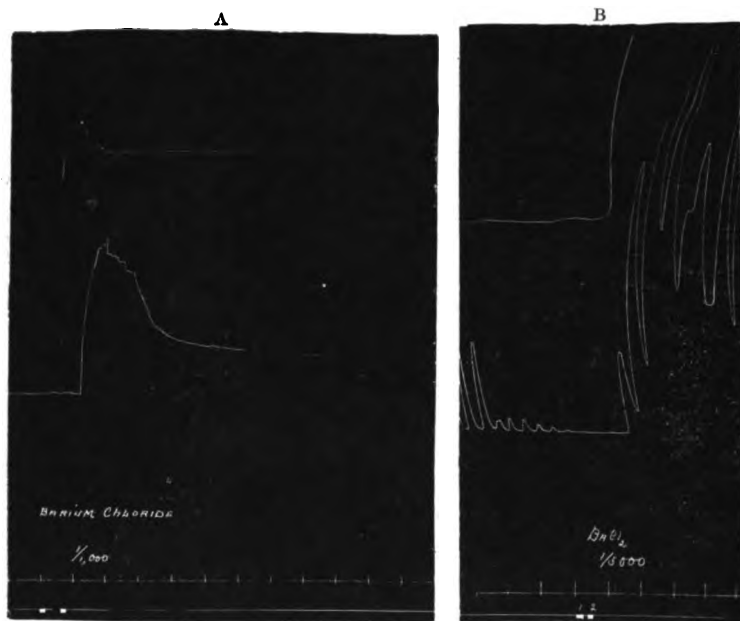


Fig. 14. Rabbit 1903. I. 1, jejunum. 2, ileum. A, reaction to barium chloride after 18 minutes in $N/1000$ zinc malate. B, 1926 reaction to barium chloride after about 18 minutes in $N/200$ acetate.

nickel acetate were employed. The reaction to barium was still present in intestinal segments treated with $N/100$ nickel acetate. The ileum exhibited a fair degree of rhythmic contractility when treated with $1/500$ $BaCl_2$ after 25 minutes suspension in $N/100$ nickel acetate, the jejunum did not show rhythmic action but tonus rose as a result of $BaCl_2$ treatment. Barium chloride produced powerful contractions after 18 minutes exposure of the intestinal segments to $N/200$ nickel acetate. The effect of $BaCl_2$ was much less when they had been acted

upon by $N/1,000$ zinc malate for 18 minutes (1903 I and 1926), thus furnishing additional evidence of a difference in the behavior of these two metals which suggest a different mechanism of their toxicity. This is also indicated by the recovery and stimulation which often occurred in intestinal segments suspended in dilute solutions of nickel acetate but which was seldom seen in experiments with zinc malate. It may be recalled that Voegtlin (21) suggested on the basis of experiments on the isolated heart that the mode of action is the same for all heavy metals.

The reaction of different parts of the intestine to zinc and nickel was found to vary considerably in some of our experiments, the ileum and colon being less readily affected than the jejunum and duodenum. Contractility often disappeared in the latter while it persisted for a considerable time in the ileum and colon. Siccardi (25), who experimented with the isolated intestine, observed that lead acetate may produce a rise of tonus in segments of the small intestine and depression in the large intestine and rectum. In experiments with chenopodium, however, the writers (26) found that the ileum is less resistant than other parts of the small intestine. The recent work of Alvarez (27) on the contractility of various parts of the small intestine, indicating that frequency of rhythm diminishes with distance from the pylorus, would explain the results we obtained with chenopodium and those of Siccardi with lead. The action of zinc and nickel on different parts of the intestine probably depends upon different mechanisms.

SUMMARY AND CONCLUSIONS

1. Zinc is a powerful depressant, exposure to very dilute solutions for 30 to 45 minutes causing decreased contractility of the intestine from which it only partly recovers on changing to pure Locke's solution.

2. Medium concentrations of zinc may completely and permanently abolish contractions.

3. Dilute solutions of nickel acetate may stimulate intestinal contractility after causing primary depression. Only the more concentrated solutions inhibit muscular activity but considerable improvement was observed when changed to pure Locke's solution.

4. That nickel is much less toxic than zinc was also shown by the reaction of the intestine to barium chloride and pilocarpine, response to these agents being obtained after treatment with a much more concentrated solution of nickel acetate than of zinc malate.

5. The reaction to barium persisted longer than the response to pilocarpine after the intestine was exposed to the influence of zinc and a concentrated solution of nickel acetate.

6. The intestine of the cat is more resistant to the action of zinc than that of the rabbit. The difference is not so marked in experiments with nickel.

7. The ileum and colon were more resistant both to the action of zinc and the depressing effect of higher concentrations of nickel.

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NERVE CONDUCTION, AND OTHER REACTIONS IN CASSIOPEA

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The rhizostomous scyphomedusa *Cassiopea xamachana* of Florida lives in shallow semi-stagnant lagoons wherein the temperature, salinity, and CO_2 are subject to considerable range. Thus it is one of the most favourable of marine animals for physiological studies, being so well adapted to aquarium conditions that the death rate is practically *nil* even though the experiments may continue for more than a month. Thus the medusa can be starved for at least 41 days in doubly filtered sea-water, its final weight becoming reduced to $1/27$ the original. The gelatinous substance of the bell is consumed during starvation, and there is no differential consumption of body substances as in vertebrates, but one and only one set of substances are consumed. Thus the loss of weight each day is proportional to the body weight at the beginning of that day, and

$$y = W(1-a)^x$$

where y is the weight at the end of x days, W is the original weight when starving began and a is a constant being usually about 0.056 (1).

The medusa can survive a concentration of carbon dioxide in the sea-water which would be fatal to most other marine animals, and it may be kept for six weeks or more in the dark, at the end of which time its commensal plant cells will have largely disappeared and the medusa loses its olive green color and appears bluish and translucent, but it remains pulsating despite the fact that its plant cells are no longer able to reduce the CO_2 produced by its activity.

Small young medusæ pulsate more rapidly than large ones, and if a small and a large one be grafted together so that the nervous network of the subumbrellas comes into contact the two medusæ pulsate in unison, the small active individual initiating every pulsation in accord with Loeb's rule that in a physiological series the rate of activity is

that of the fastest member. Hence if we pinch the larger medusa it becomes the more rapid, and the controls the movements of the complex. Also as observed by Eimer (2) and Romanes (3) and as studied quantitatively by Cary (4) the marginal sense organs which initiate each pulsation are enabled to maintain a faster rate when enervating a large than when attached to a small area of tissue. Thus the rate of the two grafted medusæ is faster than that of either when cut apart. Also Cary (5) finds that the sense organs have still another function for they hasten the initial stages of regeneration in case of injury, and this action is independent of the rate of pulsation of the medusa. Even a single one of the normally 16 or more sense-organs can maintain the rate of pulsation and hasten the early stages of regeneration, and Tashiro (6) finds that the rate of metabolism measured by CO_2 production is decidedly more rapid in medusæ with even a single sense-organ than in those pulsating without sense-organs. Evidently these marginal sense-organs exert a control over the metabolic activities of the medusa. Each sense organ is a finger-shaped hernia-like diverticulum of the general gastro-vascular space of the medusa and contains an ectodermal ocellus, and an entodermal mass of crystalline concretions. Mayer (7) finds that these concretions are a uric oxalate of calcium and they augment in mass as the medusa grows older. He therefore, believes that it is the function of these sense-organs to maintain a slight local excess of sodium at the nerve centers of the medusa, and as sodium is well known, from the work of Ringer, Loeb and others, to be a powerful stimulant its presence would suffice to produce the periodic response of pulsation.

The chemical equation in so far as the important elements, the calcium oxalate and sodium chloride are concerned, may be written as follows:



It appears that a soluble uric oxalate of sodium is being constantly manufactured in the distal end of the sense-club, and this precipitates the calcium which enters the sense-club from the outside, thus setting free a local excess of sodium chloride at the nerve center. The probability of this being the case is seen when we cut off the distal end of the sense-club, thus paralyzing the subumbrella tissue, but pulsations are restored if we flood the stump of the sense-club with a solution of 2 grams of sodium oxalate in 1000 cc. of sea-water, or with sea-water containing a corresponding excess of NaCl . Moreover when the sense-

clubs are cut off they regenerate and pulsation commences when the calcium oxalate crystals begin to form. As the formation of uric acid, urates, and oxalates is well known in animals there is nothing remarkable in this process.

Of the 16 or more marginal sense-organs, one only, the one which for the moment works the fastest, controls the rate of pulsation. The medusa normally pulsates at a slow but fairly regular rate, but an extra pulsation or a temporary quickening of the rate is commonly followed by a compensatory pause. Indeed the medusa when in systole is relatively insensitive to stimuli, and despite the fact that the pulsation is neurogenic, the differences between the activities of the medusa and those of the vertebrate heart are almost wholly of degree rather than of kind. Bethe (8) in a remarkable series of experiments makes this quite clear.

As is well known to physiologists through the work of J. Loeb and others, a slight excess of sodium, potassium, acid (H) or alkali (OH) causes the rate of pulsation to be augmented, but in greater concentration of these ions it is diminished. Thus a weak concentration of the hydrogen cation such as in carbon dioxide is a stimulant to the nerves, and causes muscular contraction, but in stronger concentration it becomes toxic and the muscles relax. Thus if there be rhythmically beating cilia overlying the muscles as in ctenophores, a slight excess of H or of OH causes muscular contraction, thus intensifying the skin-tension and pressing upon the cilia-bearing cells, and at once the cilia, being very sensitive to pressure, stop beating. Then if we increase the concentration of the acid or alkali, or prolong its action, the muscles become relaxed and the cilia relieved of pressure may pulsate at an abnormally rapid rate (9). The same effects are produced by an excess of sodium or potassium which in weak concentration stimulate nervous and muscular activity but in stronger solutions are depressant. A very different effect is produced by magnesium which is depressant in any concentration and the first effect of which is to relax the muscular tonus, thus relieving the skin-pressure upon the cilia and permitting them to beat with abnormal rapidity. The fact is that the cilia-bearing cells, although affected in the same way as are the nerves and muscles by H, OH, Na, Ca, K, or Mg, are not so sensitive and thus when freed from pressure may even appear to be stimulated, the depressant effect of the ion being more than offset by the relaxation of the skin-pressure.

As with the cilia so with the nerves and the muscles, their activities are affected in different degrees by the cations of the sea-water. Thus

magnesium depresses the activity of the muscles more than it affects the nerves,¹ but Harvey (10) finds that NaOH, KOH, Sr(OH)₂ and certain alkalies such as ethylamine and tetraethylammonium hydroxide depress the nerves more than the muscles, so that finally only slow myogenic contractions can be sent through the tissue in response to each stimulus. This however is exceptional for to most reagents the nerves are more resistant than the muscles so that if one cuts a strip of subumbrella tissue with a sense-organ left on at one end and then places the middle of the strip in 0.4 molecular MgCl₂ or 0.6 molecular MgSO₄ while the two ends of the strip are in natural sea-water, it will soon be seen that the contraction which starts at the sense-organ will pass through to the other end of the strip without any movement appearing in the middle. The same effect is produced by heating the middle of the strip to about 36° or cooling it to 12°C., or placing it in sea-water charged with CO₂; and all these experiments show that muscular response is one thing while the nervous stimulus is another, and the muscles may or may not respond to the nerve stimulus dependent upon their condition.

The present writer (11) found indeed that rhythmical pulsation could be started and maintained even when there were no marginal sense-organs. If for example we cut off all the marginal sense-organs the medusa is at once paralyzed, and responds only by a single contraction to each stimulus lapsing immediately afterwards into a state of inactivity. This was observed many years ago by both Eimer and Romanes, but they did not succeed in causing medusæ to pulsate rhythmically and spontaneously after their marginal sense-organs had been cut off. This however can be done if we first remove the sense-organs and then cut any circuit-shaped strip of subumbrella tissue. If then a contraction wave be started by mechanical, chemical, or electrical stimuli so that it goes in *one* direction it cannot escape from the circuit of tissue and being entrapped must continually pass through it at a practically uniform rate, provided the chemical and physical conditions of the environment remain unchanged. Harvey (12) found that such a contraction wave may course for eleven days through the tissue with no appreciable decline in rate, traveling 457 miles during this time, the average rate of nerve conduction being 440 mm. per second at 28.9°C. Mayer found that such contraction waves in tissue isolated from the central nervous system could be produced in other medusæ such as *Aurellia*, *Dactylometra*, *Cyanea*, *Rhizostoma pulmo* or *Cotylorhiza*,

¹ The reverse is the case in mammals, as shown by Meltzer and Auer, and Joseph.

and also in ring-shaped strips cut from the ventricle of sharks, or the loggerhead turtle (13). Later Garrey (14) gave a good description of the same phenomenon in the ventricle of the loggerhead turtle evidently being unaware of the previous experiments made at Tortugas.

It is only necessary that the length of the circuit should be long enough to permit a sufficient interval of rest before the return of the entrapped wave. Even so a ring of subumbrella tissue pulsates, according to Cary (15), about three and one-half times as rapidly as does the normal medusa activated by its sense-organs, and Mayer found that it could respond by a contraction to each and every induction shock even when the shocks came at double this rate. We see therefore that the medusæ pulsate normally at only about one-seventh the rate they are capable of maintaining. As is well known tissue which has been in pulsation is thereby exhausted and must rest for a time before it can again respond to a constantly present stimulus. Thus rhythmical pulsation represents alternate fatigue and recovery.

When medusæ are pulsating normally with sense-organs intact a sudden shock or any very strong stimulus may cause the major part of the wave from the activating sense-organ to go to one side instead of spreading equally over the disk, and this starts a circuit wave which rushes around and around the disk at about two or three times the normal rate of the jellyfish. This shows that the mechanism designed to prevent the exhausting condition of an entrapped wave is imperfect. In fact the pulsation waves normally arise from one sense-organ, the fastest working one, and travel around the ring of subumbrella tissue in both directions "setting off" each successive sense-organ as the waves pass. Finally the two waves meet "front to front" on the opposite side of the medusa and being equal each to each annul one the other. If however one of these waves is more intense than the other it will overpower the weaker wave and then travel constantly around the circuit until interfered with by some other wave which advances against it, or until it is obstructed in the nerve net or the exhausted tissue can conduct it no longer. The muscles tire much more rapidly than the nerves and after an entrapped wave has gone for two or three days the muscular movement is barely discernible, but the nerves may conduct for a week or more maintaining a practically constant rate.

In the vertebrate heart the mechanism to prevent entrapped waves is much more perfect for here, as is well known, each pulsation arises in the region of the sinus venosus, then spreads over the auricles and finally into the ventricle. In the loggerhead turtle it travels in the compact external muscular layer of the heart, the cavernated interior

being passively squeezed as if it were a sponge. In fact the cavernated interior can be cut away and the peripheral muscular layer can still maintain a circuit wave if cut into the form of a ring and then activated by a single induction shock. No circuit wave can arise in nature for the pulsation normally dies out at the apex of the ventricle and cannot return over the old path, for the tissue must rest before it can again respond.

It is fortunate for physiological studies that we can maintain these entrapped circuit waves in tissues, for this enables us to study a *single* stimulus which in the case of the medusa maintains itself without apparent diminution for days. The stimulus is neurogenic in the medusa and is thus not easily exhausted, but in the vertebrate heart it is myogenic and in this case fatigue soon develops and the circuit waves soon die out, although Garrey (16) succeeded in maintaining such a wave in the ventricle of the loggerhead turtle for seven hours.

As is well known from the studies of the brothers Hertwig (17), and of Hesse (18), the nerves of scyphomedusæ form a network immediately under the ectodermal epithelium of the subumbrella and overlying the muscles. The nerve cells are usually bipolar and the fibers are non-medullated. In *Cassiopea* the subumbrella alone responds to stimuli, the exumbrella being so poorly supplied with nerves that its depressed centre may be flooded with corrosive sublimate without producing the least effect upon the regular rhythm of the medusa, provided the poison does not diffuse into the surrounding sea-water and thus reach the sense-organs and the subumbrella. Of all parts of the medusa the marginal sense-organs or rhopalia are most sensitive to stimuli, but even a ring which lacks sense-organs and is pulsating by means of an entrapped wave will respond by suddenly augmented muscular contractions when locally stimulated in any manner.

We must keep clear in our minds that under normal conditions the marginal sense-organs or rhopalia initiate neurogenic stimuli, and that this nervous stimulus secondarily affects the muscles causing them to respond, but the nervous stimulus can be made to pass through the subumbrella without causing the least visible response from the muscles, and conversely as found by Harvey the muscles may under certain conditions transmit a slow myogenic contraction even when the nerves have ceased to function.

The muscles are much more affected by temperature, salinity, or excess or deficiency of sodium, potassium, calcium and magnesium than are the nerves. For example if the medusa be heated from 29° to 38°C.

the muscular movement is barely perceptible but the nerves now conduct at about one and one-half times their former rate. Similarly if the sea-water be diluted by mixing 33.3 parts of sea-water with 66.6 parts by volume of distilled water the nerves still conduct at about 29 per cent the normal rate but the muscular movement is so reduced that it is usually impossible to record it on a kymograph. Also the muscular movement is at once brought to a stand-still by placing the medusa in 0.4 molecular magnesium chloride or 0.6 molecular magnesium sulphate but the nerves still conduct but with diminishing velocity. If the sea-water be diluted with 0.4 molecular magnesium chloride the decline in rate of nerve conduction is nearly the same as if we diluted the sea-water with distilled water as shown in the following table. This shows the remarkable insensibility of the medusa to changes in osmotic pressure.

TABLE I

COMPOSITION OF THE SOLUTION	A AVERAGE RATE OF NERVE CONDUCTION IN SEA-WATER DILUTED* WITH DISTILLED WATER	B AVERAGE RATE OF NERVE CONDUCTION IN SEA-WATER DILUTED WITH 0.4 MOLECULAR $MgCl_2$ DISSOLVED IN THE SAME DISTILLED WATER AS USED IN COLUMN A
Natural sea-water.....	100	100
95 cc. sea-water + 5 cc. distilled water.....	100.5	97.9
90 cc. sea-water + 10 cc. distilled water.....	95.89	95.3
80 cc. sea-water + 20 cc. distilled water.....	88.3	88.9
70 cc. sea-water + 30 cc. distilled water.....	81.4	78.1
60 cc. sea-water + 40 cc. distilled water.....	71.1	67.2
50 cc. sea-water + 50 cc. distilled water.....	56.31	55.4
33.3 cc. sea-water + 66.7 cc. distilled water.....	29.	

* This distilled water contained a slight amount of CO_2 and was thus acid and stimulating.

The hydrogen ion in weak concentration such as $1 n \times 10^{-5}$ stimulates both muscular contraction and the rate of nerve conduction, the effect upon the muscles being more marked than upon the nerves, but in greater concentration acids soon become toxic and depress the activity of both muscles and nerves, especially the muscles; and it is interesting to see that Osterhout (19) presents a table showing that in plants a weak concentration of HCl is at first stimulating but later after a greater concentration of the H ion has passed through the plasma membrane of the plant it becomes depressant.

Amines and inorganic hydrides (20) are stimulating in weak concentration but as shown by Harvey (21) certain of them in stronger concentration become more depressant to nerve conduction than they do to muscular response so the muscles can still transmit a myogenic contraction after the nerves cease to function. Also I find that if sea-water be diluted with alkaline distilled water containing $0.75n\ 10^{-6}\ OH$ ion concentration the muscles still contract vigorously even in 50 per cent sea-water and 50 per cent of this distilled water, whereas in neutral or slightly acid distilled water the muscular response would be very feeble in this solution.

An interesting selective effect is produced by calcium. If the normal medusa be placed in an artificial sea-water² solution which however lacks calcium it ceases to pulsate for one to six minutes and the pulsations thereafter are only occasional and are separated by longer and longer intervals of time so that after a few hours all pulsation ceases and the medusa remains relaxed and inert; but even after a day or two of quiescence it can be almost instantly activated by restoring the calcium. Howell as is well known observed the same phenomenon in the turtle's heart in 1901.

Nerve conduction is, however, affected but little by the absence of calcium in the surrounding sea-water, the calcium of the tissues being sufficient to maintain a nearly normal rate in an entrapped wave for several hours.

A more striking phenomenon is seen when the medusa is placed in a partial sea-water solution which lacks magnesium,³ or in sea-water containing an excess of calcium. After a few hours the medusa goes into clonic tetanus (22), and the muscles finally tear themselves into shreds; but even after 24 hours recovery of normal tonus and pulsation is very rapid if we add sufficient magnesium; for the Mg cation being depressant in all concentrations relaxes the muscular tonus (23). Calcium tetanus is purely muscular and local for if part of a strip of tissue be dipped beneath the surface of sea-water containing an excess of calcium it develops a local tetanus which does not spread to those parts of the

81.1	volumes	0.6	molecular	NaCl
14.36	volumes	0.4	molecular	MgCl ₂
2.84	volumes	0.39	molecular	CaCl ₂
1.7	volumes	0.6	molecular	KCl

Total 100.00

² A solution which resembles sea-water but lacks MgCl₂ would be made up by omitting MgCl₂ in the above solution.

strip which remain in normal sea-water. Moreover this tetanus cannot take place unless sodium as well as calcium be present for if we place a medusa in a pure calcium solution or in one containing the proportions of calcium, potassium and magnesium found in sea-water no tetanus develops. Thus there is reason to support the contention that sodium and calcium combine or become somehow intimately associated with each other in these reactions. In 1906 the present writer (24) observed that in medusæ calcium assists the sodium to overcome the inhibiting effects of magnesium, and this was discovered to be true by Blake and by Meltzer and Auer for mammals, and by Mines (25) for fishes. J. Loeb was the first definitely to determine that calcium ions can produce tetanus in muscle, but he went further and found in 1902 that the salts of monivalent cations exert toxic effects, but that these effects can be counteracted by slight amounts of salts with bivalent cations. Thus a weak concentration of the calcium ion can annihilate the toxic effect of a strong concentration of the sodium ion (26). Osterhout (27) shows also that a similar antagonism between the Na and Ca ions exists in plants, and that salts are also antagonized by acids; a condition first observed in animals by Loeb. So this relation is apparently general for all organisms.

Recently Loeb (28) finds that if the chlorides of sodium, potassium, calcium and magnesium be mixed in proportions found in sea-water and if experiments be made with solutions ranging from $\frac{1}{4}$ m. to $\frac{1}{2}$ m. the mixtures which maintain a normal activity in *Balanus* larvæ are those in which the concentration of the NaCl + KCl is about 35 times as great as the CaCl₂ + MgCl₂. "In other words the concentration of CaCl + MgCl required increases in direct proportion with the concentration of NaCl + KCl, thus following Weber's law."

But to return to the special consideration of *Cassiopea*, we see that its muscular activity is stimulated by the combination of sodium, calcium and potassium found in sea-water, but inhibited by the magnesium so that only a normal muscular tonus remains as a result. The muscles are therefore not stimulated into activity by the sea-water as a whole, but each pulsation arises as a result of a constantly present neural stimulus, probably sodium, in the marginal sense-organs. Thus the muscular tissue of the jellyfish is in a balanced medium containing stimulants, Na + K, and a depressant, Mg, which antagonize one the other. The Ca while itself a depressant becomes a sustainer of activity when combined or associated with the sodium.

For the nerves the conditions are quite different for we find that the decline in the rate of nerve conduction in diluted sea-water is nearly the same whether we dilute with distilled water, 0.9 molecular dextrose, or 0.4 molecular magnesium chloride. In other words the Mg ion is nearly as neutral as is distilled water in respect to nerves, but for muscular activity it is a decided depressant. The fact that both distilled water and 0.9 molecular dextrose produce each a similar effect upon the nerves when used to dilute the sea-water shows that osmotic pressure has little effect upon the nervous activity of the medusa (30).

It is also important to observe that magnesium takes practically no part in the control of the rate of nerve conduction, being nearly as inert as distilled water in this respect. The concentration of the sodium, calcium and potassium cations however determines the rate of nerve conduction. For example if we dilute the sea-water with distilled water which is nearly neutral but still slightly alkaline with an OH concentration between 10^{-6} and 10^{-7} the rate of nerve conduction in *Cassiopea* declines as follows: If y be the rate of nerve-conduction and x be the concentration of the sodium, calcium, and potassium cations in the surrounding sea-water.

$$y = 2.512 x^{0.8} \quad \text{and} \quad \frac{x^{0.8}}{y} = 0.398$$

$$\text{or } \log y = \log 2.512 + 0.8 \log x.$$

This is identical in form with Freundlich's formula for *adsorption*, and suggests that the sodium, calcium and potassium cations are adsorbed and that these adsorbed cations conduct the nerve stimulus, the rate of which is by Wilhelmy's law proportional to the concentration of the cations which conduct it (31). As we have before observed if OH or H ions be present they will accelerate the rate of nerve conduction, but the significant fact appears to be that Na, Ca, and K are alone *sufficient* to conduct the nerve stimulus. In nature however there are probably always some free OH ions in sea-water and these would accelerate the rate. For example if we dilute the sea-water with alkaline distilled water having an OH concentration of $0.75 \text{ m} \times 10^{-5}$ the decline in rate is as follows:

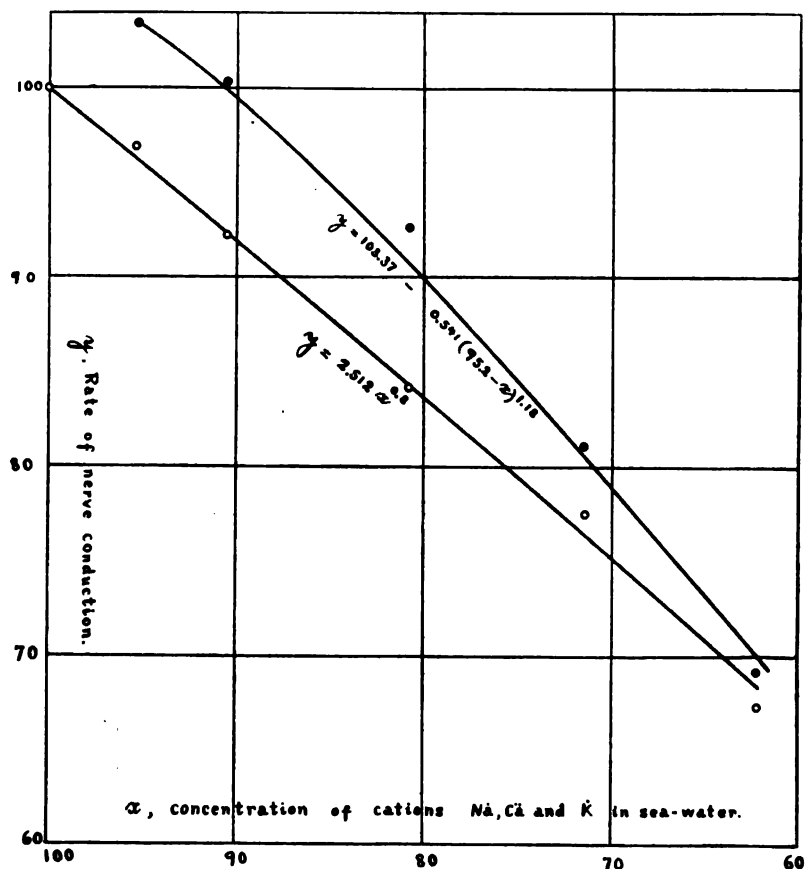


Fig. 1

We see that the OH anion in weak concentration, such as $6 m \times 10^{-5}$, is highly stimulating to the rate of nerve conduction but that it becomes depressant in concentration higher than this. Moreover when OH is present in this concentration the formula for the rate of nerve conduction cannot be stated by the simple expression $y = a x^{\frac{1}{n}}$, but it assumes the form $y = B - d (C - x)^p$ where B is the highest rate of nerve conduction observed, C is the relative concentration of the cations Na, Ca and K corresponding to this highest rate and p is an integral exponent. When the distilled water is nearly neutral, however, as in Table II the equation for the rate of nerve conduction can be written

TABLE II

Rates of nerve conduction in Cassiopea in sea-water diluted with slightly alkaline distilled water

COMPOSITION OF THE SOLUTION	\bar{x} RELATIVE CONCENTRATION OF THE Na, Ca, AND K CATIONS IN THE SEA-WATER	y OBSERVED RATES OF NERVE CONDUCTION. (OBSERVED y)	RATE CALCULATED FROM THE FORMULA $y = 2.61\bar{x}^{0.4}$	THE RATIO $\frac{\bar{x}^{0.4}}{\text{OBSERVED } y}$	RATE CALCULATED FROM THE FORMULA $y = 100 - 0.778 (100 - \bar{x})^{1.49}$
Natural sea-water.....	100	100	100	0.398	100
95 cc. sea-water + 5 cc. of nearly neutral distilled water.....	95.2	96.86 \pm 0.66*	96.15	0.395	96.15
90 cc. sea-water + 10 cc. of nearly neutral distilled water.....	90.5	92.25 \pm 1.62	92.32	0.397	92.29
80 cc. sea-water + 20 cc. of nearly neutral distilled water.....	80.8	84.2 \pm 2.15	84.27	0.398	84.21
70 cc. sea-water + 30 cc. of nearly neutral distilled water.....	71.4	77.6 \pm 1.82	76.38	0.397	76.29
60 cc. sea-water + 40 cc. of nearly neutral distilled water.....	62.2	67.5 \pm 2.14	68.40	0.403	68.48

* Probable errors are stated as \pm following a determination.

TABLE III

COMPOSITION OF THE SOLUTION	\bar{x} RELATIVE CONCENTRATION OF THE Na, Ca AND K CATIONS OF THE SEA-WATER	y OBSERVED RATE OF NERVE CONDUCTION	RATE CALCULATED FROM THE FORMULA: $y = 103.37 - 0.541 (95.2 - \bar{x})^{1.45}$
Natural sea-water.....	100	100	
95 cc. sea-water + 5 cc. of alkaline distilled water.....	95.2	103.37	103.37
90 cc. sea-water + 10 cc. of alkaline distilled water.....	90.5	100.33	100.00
80 cc. sea-water + 20 cc. of alkaline distilled water.....	80.8	92.67	90.78
70 cc. sea-water + 30 cc. of alkaline distilled water.....	71.4	81.09	80.59
60 cc. sea-water + 40 cc. of alkaline distilled water.....	62.2	69.33	69.87

in either form and $y = a x^{\frac{1}{n}} = B - d (C - x)^p$ or in the case of Table II

$$y = 2.512 x^{0.8} = 100 - 0.778 (100 - x)^{1.019}$$

Thus the H or OH ions when present are not adsorbed but act as independent stimulating ions.

We venture to suggest that adsorption may play a fundamental rôle in nerve conduction and that the sodium, calcium, and potassium cations are attracted to the surfaces of negatively charged colloidal particles, for the sea-water and fluids surrounding the nerves being alkaline the colloidal elements of the nerve may be expected to carry a negative charge (32).

The number of the cations which the colloidal particles can capture and adsorb must depend upon the magnitude of the negative charges on the particles, and also upon the concentration of the cations in the surrounding fluid which in this case is sea-water.

A series of diagrams may serve to illustrate this hypothesis. Thus in figure 2 the nerve is represented by a row of negatively charged colloidal particles, for the colloid being normally alkaline the charge may be assumed to be negative. Line No. 1 shows the nerve in its resting stage wherein the negative charge of each colloidal particle tends to be partially neutralized by the adsorbed cations of sodium, calcium and potassium shown by + + +. The number of cations which each colloidal particle can capture and temporarily de-ionize (33) depends upon the potential of its negative charge and also upon the concentration of the cations in the surrounding fluid. For the sake of illustration we have shown three such cations attracted to the surface of each particle, but in reality the number must be greater than this.

Line No. 2 shows the beginning of a nerve impulse wherein the adsorbed cations of particle (A) have combined with some anions to form an ion-proteid, thus losing their positive charges and unmasking the negative charge of the colloidal particle. As a result other cations from the surrounding fluid (sea-water) are at once attracted and captured by the particle.

In Line No. 3 the reaction has passed on to particle (B) and its negative charge is unmasked, and thus the negative charge passes through the nerve at the rate of nerve conduction until each particle has lost its original cations, and then recaptured others from the surrounding fluid as in Lines 2-6, Line 6 representing the resting nerve after the reaction has passed through it.

Since 1899 Loeb (34) has maintained that physiological reactions are chemical phenomena associated with the formation of ion-proteids, but I think that while this is true for nerve conduction, it is only half the truth and that it is possibly a phenomenon of *adsorption* combined with that of an ordinary chemical reaction.

Loeb indeed is not antagonistic to the view that complex changes other than those of a simple chemical reaction may accompany nerve conduction, for he says: "We have to remember that all life phenomena are due to motions or changes occurring in colloidal substances" (35). No one however had reason to support the view that adsorption plays

a part in nerve-conduction until the discovery of the change in rate of nerve conduction in *Cassiopea* in successive dilutions of sea-water suggested this to me as a possibility.

My results lend no support to the theory of Sutherland (36) that the velocity of propagation of nerve impulse is that of a shear in the substance of the nerve. If this were the case its rate would vary with the viscosity of the surrounding fluid, but the decline in rate is practically the same whether the sea-water be diluted with distilled water, 0.9 molecular dextrose, or 0.4 molecular magnesium chloride.

Matthews (37), 1902, states that protoplasm consists essentially of a colloidal solution, and stimulation is accompanied by the passing of this solution to or toward a gel; and with these statements I am in accord. Matthews, however, believed the anions to be the stimu-

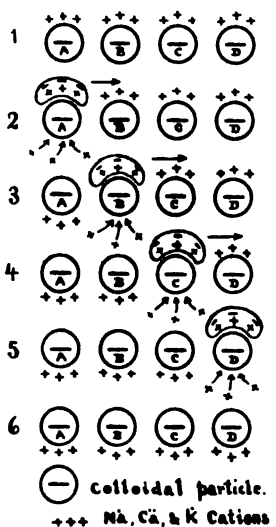


Fig. 2

lating ions, and he also thought the colloidal particles carry a positive charge. Later studies by many students have made it apparent that the cations are the more active agents in most physiological reactions, and that living protoplasm is normally alkaline and thus its colloids probably carry negative charges. Moreover the phenomena of adsorption were not well understood in 1902 and Matthews makes no mention of it in respect to nerve conduction.

It will be recalled that Harvey (38) showed that in *Cassiopea* the rate of nerve conduction augments in a right-line ratio as one heats the medusa from about 17° to about 35°C. beyond which it suddenly declines, and this result has been confirmed by Mayer (39). Also Knowl-

ton and Starling (40) found a right-line ratio for the excised hearts of dogs and cats when heated from 24° to 40°, the increase in rate being arithmetically proportional to the increment of temperature. Now if these were simple chemical reactions the rate should augment with rise in temperature in accord with the Van't Hoff law,

$$y = b (C)^z$$

where C is a constant usually from 2 to 3, and Z represents intervals of 10°C. Thus if at n° , $Z = 1$; at $(n + 10)^\circ$, $Z = 2$; and at $(n + 50)^\circ$, $Z = 5$, etc.

This law, which is based upon the previous work of van't Hoff for the speed of chemical reactions at ascending temperatures, was studied in detail by Snyder (41) for about 20 physiological activities and found to hold. It is therefore remarkable that it does not hold for the neurogenic pulsation wave of the medusa and for the myogenic activity of the vertebrate heart, for in both these reactions the rate augments more slowly than would be expected from Snyder's studies. If, however, the Na, Ca, and K cations which are essential to the reaction are adsorbed we would expect the rate to be reduced, for as Bayliss (42) points out heat dissociates an adsorption compound in a linear ratio with rise in temperature. Thus less is adsorbed at a high than at a low temperature.

In fact surface tension which is so intimately associated with capacity for adsorption also has a negative temperature coefficient, and it seems possible that some such factor may account for the observation of Weizäcker (43) that heat production in muscles is higher the lower the temperature, and muscular tension also has a negative temperature coefficient.

According to Bayliss the amount of substance adsorbed declines in a linear ratio of about 4.5 per cent for each 10° rise in temperature. At 17.5°C. the average rate of nerve conduction in *Cassiopea* is 54.5, and at 27.5° it is 103, instead of 152.5 as it would be did it follow Van't Hoff's law $y = 54.5 (2.8)^z$, and it is reasonable to suppose that if nerve conduction depended solely upon the OH ion as the catalyzer of a chemical reaction its rate would be not far from 152.5. If however it depended solely upon the adsorbed cations Na, Ca, and K its rate might be expected to be $54.5 - 0.045 \times 54.5 = 52.05$ and the resultant rate would be $\frac{152.5 + 52.05}{2} = 102$ which is close to the observed 103.

At temperatures higher than 33° heat depression (44) becomes a

factor and must seriously depress the rate of all metabolic activities. Thus the rate of nerve-conduction must soon reach a maximum and then decline rapidly as observed by Harvey. Thus the facts of temperature-reaction appear to accord with the hypothesis that the sodium, calcium and potassium cations are adsorbed by colloidal elements of the nerve.

It will be recalled that Lewis (45) found that inorganic salts lower surface tension at a water-hydrocarbon interface, hence such an adsorption as the one I postulate is possible; moreover the exponent 0.8 in the equation $y = 2.512x^{0.8}$ is not that of a partition coefficient for in this case if the exponent were stated as a vulgar fraction the denominator would be a simple integer. The exponent 0.8 is high for adsorption phenomena, but Mecklenburg (46) cites 16 cases of adsorption in which the exponent ranges from 0.167 to 0.965, being usually between 0.2 and 0.6. Hence 0.8 is not beyond the range of known cases of adsorption.

The question arises whether physiological activities other than nerve conduction may not be determined or influenced by adsorption. Goldfarb (47) has studied this question in relation to regeneration and finds that if we dilute the sea-water with distilled water which is acid with H_2CO_3 the rate of regeneration bears a general resemblance to that for nerve conduction when the sea-water is diluted with slightly acid distilled water. Later Goldfarb (48) found the same general curve for dilution with alkaline sea-water, but until his experiments have been completed and he has diluted the sea-water with neutral distilled water he prefers not to draw conclusions. Goldfarb's results support those of Loeb (49) that regeneration is somewhat more rapid in slightly diluted sea-water than in natural sea-water, but we do not know whether Loeb's distilled water was acid, neutral, or alkaline. *Cassiopea* is an exceptionally favourable animal for studies upon regeneration and Stockard (50) found that it conforms to Morgan's law that the deeper the level of an injury the more rapid the regeneration, for cuts made near the centre of the disk regenerate more quickly than those near the periphery. Stockard also found (51) that the regenerating tissue could develop at the expense of the body substance of the animal as in cancer, and in this he is in accord with King (52), who found that starfishes regenerate at the same rate whether starved or fed. In fact *Cassiopea* is an exceptional animal in that it is relatively insensitive to CO_2 , salinity, and temperature changes, and thus hardy in confinement, but in all known physiological reactions it differs only in degree not in kind from other marine animals.

SUMMARY

It seems probable that sodium, calcium, and potassium cations are attracted by adsorption to the surfaces of negatively charged colloidal particles of the nerve.

When a stimulus passes these adsorbed cations combine chemically with proteid elements, thus neutralizing their electrical charges and revealing the negative charges of the colloidal particles.

Thus the negative charge passes through the nerve at the rate of nerve conduction; but it is quickly neutralized, for other sodium, calcium, and potassium cations are at once attracted to the surfaces of the colloidal particles, and thus in its resting stage the nerve is nearly neutral.

OH and H ions are not adsorbed, but if present in weak concentration they accelerate the rate of nerve conduction. In greater concentration they become depressants, the H being more toxic than OH in this respect. The presence of OH or H is not necessary to nerve conduction, and when free H and OH ions are absent the rate of nerve conduction is proportional to the concentration of the adsorbed sodium, calcium, and potassium cations.

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THE EFFECTS OF TESTICULAR TRANSPLANTS UPON VASOMOTOR IRRITABILITY

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In a previous paper (1) it was pointed out that changes in functional as well as morphological processes follow the removal of the primary reproductive glands in male animals. It was further shown that blood pressure reactions to nicotin were constantly lowered as a result of gonadectomy; hence, the effects of castration were upon the sympathetic nervous system proper. At that time preliminary studies indicated that the presence of testicular grafts in such animals resulted in renewed activity of the vasomotor mechanism. This paper presents further data and conclusions on this point.

In the present, as in the former work, vasomotor reaction was taken as the criterion of the activity of the sympathetic nervous system, nicotin being used as a stimulant because of its selective action upon the ganglia (2) and vasomotor center proper (3). The pressure determinations were obtained from the femoral artery. Standard doses of nicotin, 1 cc., 1:2,000 solution, were flushed into the femoral vein with 0.9 per cent saline solution. The wounds were closed with continuous sutures and dressed with collodion cocoons. Ether anesthesia, administered by the open cone method, was used unaccompanied by other narcotics. The implants, which consisted of thin slices of partially decapsulated testicular tissue, were placed in a pocket formed among the deep dorsal muscles of the neck near the shoulder. The neck incision was found advantageous because of the ease of obtaining a deep muscular pocket for the graft, and of maintaining bandage dressings. The nature of the experiments necessitated a series of three operations upon each dog as follows:

1. The blood pressure and vasomotor reactions to nicotin were obtained on the normal dog, which was then completely castrated.
2. From 6 to 8 weeks later a second determination was made and a testicular graft placed at that time or shortly afterwards.

3. These implants were allowed to remain from 7 to 22 days, at the end of which time a third series of observations was made.

The following results and conclusions are based upon 87 blood pressure and vasomotor reactions to nicotin obtained from 20 anesthetized dogs. The numerical determinations are given in Table 1, and the average results shown graphically in figure 1.

TABLE I

Showing blood pressure, pressor effects of constant doses of nicotin and body weight determinations from three series of animals, before and after castration and after the reception of a testicular transplant. In series A are given the actual numerical readings of 9 dogs. Figures shown for Series B and C are averages computed from 33 readings made upon 11 dogs of a former work. Average results and per cent variations of Series A, B and C are based upon 87 determinations from 20 anesthetized dogs. All pressure readings are expressed in millimeters of mercury, body weight in kilos.

SERIES.....	A										B	C	AVERAGE	VARIATION
DOG NUMBER.....	1	5	6	7	8	9	11	14	15	6 dogs	5 dogs			
Nicotin 1 cc. of 1:2,000 solution.														per cent
Normal.....	90	53	57	58	60	44	46	×	×	26	44	53.1		
Castrated.....	27	20	26	22	22	64	10	26	32	17	25	26.5	-50.1	
Grafted.....	86	42	31	×	28	44	17	45	41	×	35	41.1	+55.1	
Blood Pressure Mm. of Hg.														
Normal.....	146	176	146	168	132	154	129	×	×	118	116	142.8		
Castrated.....	138	153	110	168	139	151	145	152	98	111	114	134.5	-5.8	
Grafted.....	146	174	110	×	138	155	148	150	98	×	114	137.0	+1.8	
Weight in Kilos														
Normal.....	9.55	8.64	15.90	9.55	10.12	6.82	5.00	7.84	7.27	×	×	8.95		
Castrated.....	11.82	9.55	10.46	12.10	11.68	7.82	6.25	7.96	7.07	×	×	9.41	+5.7	
Grafted.....	11.93	10.01	11.36	×	11.82	8.41	6.59	7.96	7.28	×	×	9.42		

As has been pointed out responses of the vascular system to injections of nicotin give evidence that one of the effects of castration is the lowering of irritability of the sympathetic nervous system. The entire series of 20 dogs, after castration, gave vasomotor responses to nicotin which averaged 50 per cent lower than reactions to the same drug before the removal of the gonads. That is, the normal responses to nicotin of 53 mm. of Hg. dropped to 26 mm. after gonadectomy. If this loss of irritability is brought about by the absence of the testicles, reinstatement of the missing parts should, at least, partially relieve the depres-

sion. Ten days after reception of the graft the vasomotor readings to nicotin, which at the end of 6 to 8 weeks after castration averaged 26 mm., rose to 41 mm., an increase of 55 per cent or a return to 77 per cent of the normal. According to these figures there remains a depression of 23 per cent below normal notwithstanding the presence

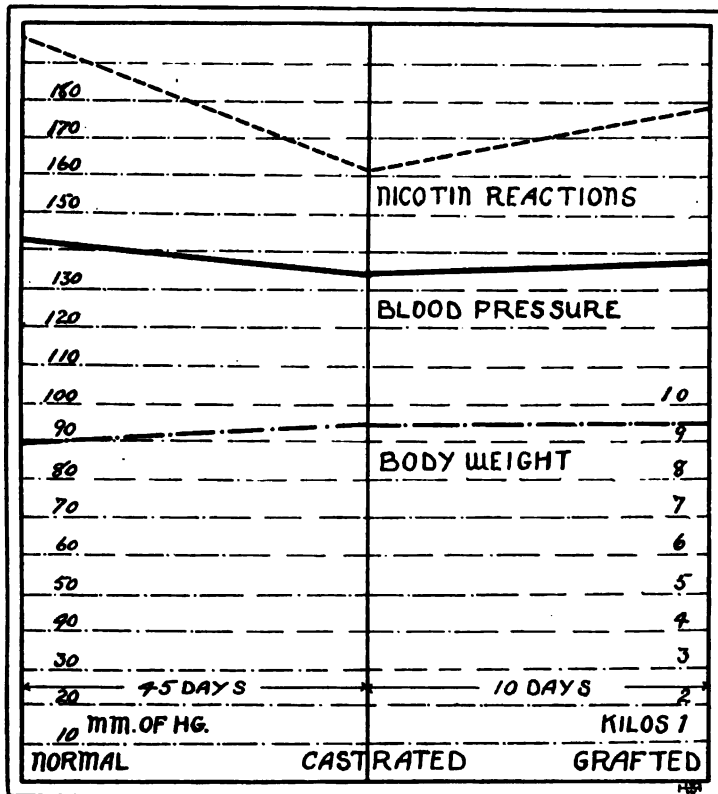


Fig. I. Composite curves of Series A, B and C showing the average blood pressure, vaso-motor responses to nicotin, and body weight of normal, castrated, and grafted dogs. Blood pressure readings are expressed in millimeters of mercury, body weight in kilos.

of the implanted materials. This condition may be due to a slow absorption of, or a deficiency of, the secretions because of the relatively small amount of testicular tissue present.

The tracings of figures No. II and III clearly show the effects of castration and the subsequent translocation of testicular material

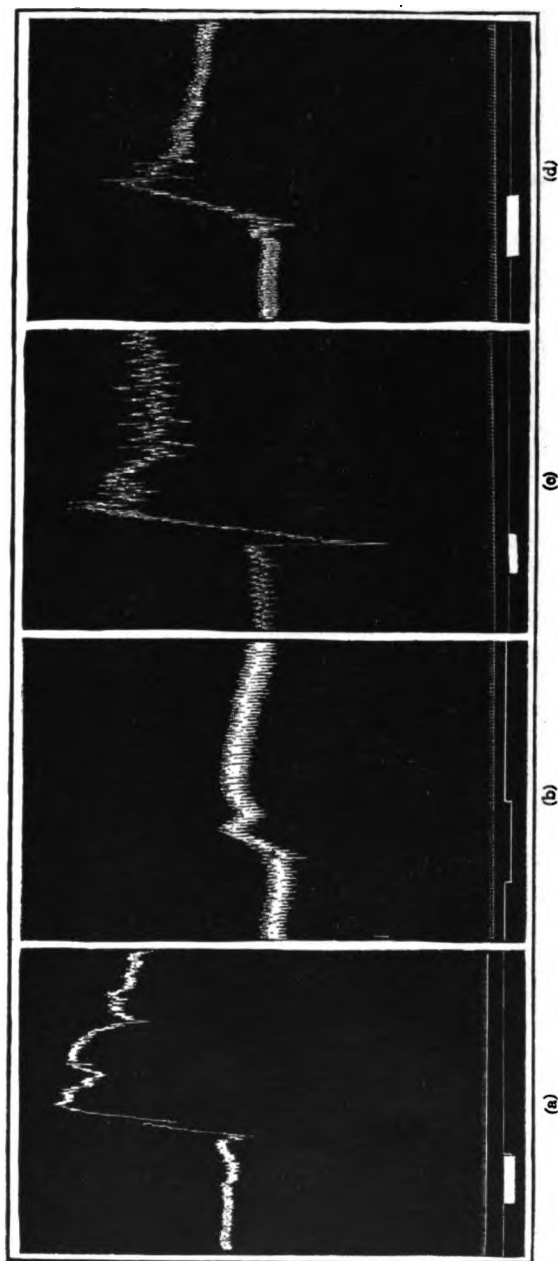


Fig. 11. Reactions of Dog No. 1, Series A, to 1 cc. of 1:2,000 nicotine solution. a, March 2, 1915, before castration; b, April 17, 1915, after the operation; c, April 29, and d, May 6, 1915, after the reception of a testicular graft. The transplant was made immediately after having obtained reading b. Time, one second.

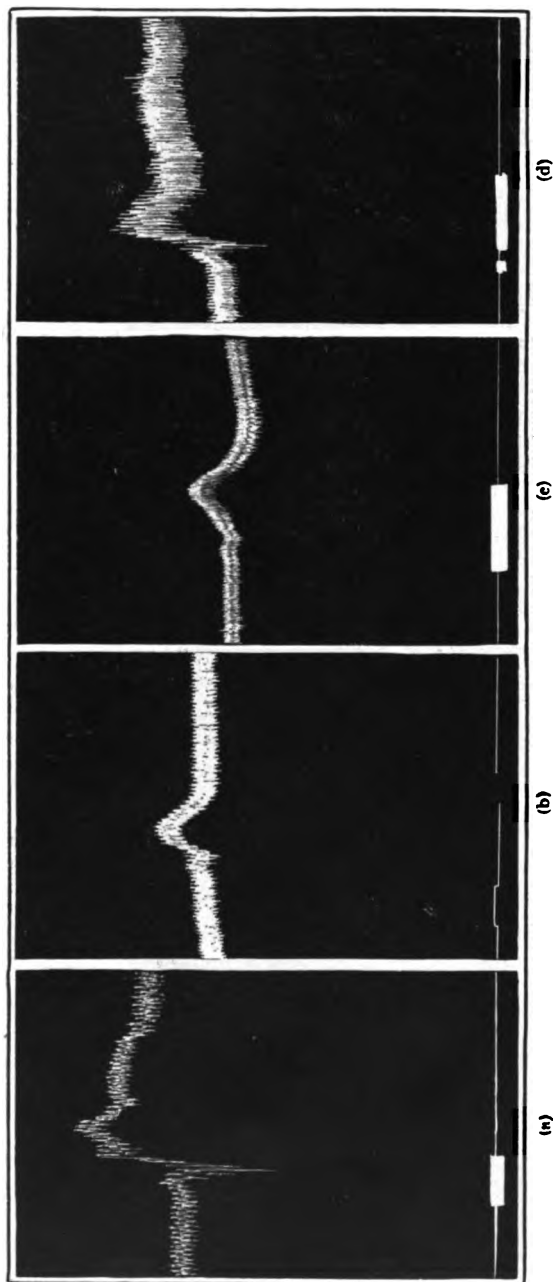


Fig. III. Reactions of Dog No. 5, Series A, to 1 cc. of 1:2,000 nicotine solution. a, March 3, 1915, before gonadectomy; b, April 20, and c, May 1, 1915, after the operation, and d, May 23, 1915, 22 days after the reception of a testicular transplant placed May 1, 1915. Time, one second.

upon the blood pressure and vasomotor reactions to nicotin. Tracing (a) of figure II, taken March 2, 1915, shows an initial blood pressure of 146 mm. and a pressor response to nicotin of 90 mm. Forty-six days after castration-tracing (b)—the blood pressure had fallen to 138 mm., 8 mm. below normal, and gave a nicotin reading of only 27 mm. On April 29, twelve days after the reception of a graft, the blood pressure, as shown in tracing (c), had returned practically to normal and gave a nicotin reading of 86 mm. Tracing (d), taken May 6, shows the continued effects of the implanted tissue. In figure III are shown four tracings from dog No. 5. Graph (a) is the reading from the normal dog. Tracings (b) and (c), both post-castration readings, show the continued lowered reactions to nicotin. Graph (d) shows the tracing obtained 22 days after the reception of a testicular transplant. The return of irritability in this case is apparent. These two figures show that the lowered reactions obtained by castration can be and are raised by the implantation of the secretory parts of the testis. So far neither the physiological life nor the period over which a graft can influence the nervous system has been determined, but our results show that they remain active for at least 22 days.

The slight fall in blood pressure following castration is easily within experimental error, however, this finding is constant and may be considered a result of the lowered activity of the vasomotor mechanism. The average normal blood pressure of 142.8 mm. of Hg. fell to 134.5 after gonadectomy, a lowering of 5.8 per cent, and was raised to an average of 137.0 mm. as a result of the presence of testicular transplants. Therefore, the presence of such implants in castrated dogs, while greatly increasing the vasomotor responses to nicotin, cause but little rise of the lowered blood pressure.

The present series of dogs showed an average increase of 5 per cent in body weight during the period of castration.

The present findings point to the conclusion that a direct relationship does exist between the internal secretions of the testis and the sympathetic nervous system. Castration results in a depressed activity of the nervous mechanism while subsequent establishment of the lost parts tends to lift the depression and at least partially to reinstate normal activity.

NOTE. The interesting observation was made during the work that normal dogs giving feeble nicotin reactions subsequently proved unusually susceptible to infections.

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STUDIES IN BLOOD PRESSURE ESTIMATIONS BY INDIRECT METHODS

I. THE MECHANISM OF THE OSCILLATORY CRITERIA¹

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INTRODUCTION

In 1901 and 1903 (1, 2, 3) the author reported the results of a series of experiments planned with the object of elucidating and evaluating some of the criteria employed in the estimation of the blood pressures in arteries by indirect methods. The introduction of new criteria for the indirect determination of the arterial pressures, the questions and the difficulties that have arisen in regard to the interpretation of the new as well as of the old criteria since then, have induced him to again turn his attention to this subject. During the past year experiments have been devised with a view not so much toward determining the accuracy of any particular criterion in the estimation of the blood pressures as toward the acquisition of more knowledge in regard to the principles underlying the criteria, to the relation of the criteria one to the other, and to the conditions modifying these relations.

We first turned our attention to certain of the moot questions bearing the pressure oscillations yielded by an artery to compression exerted from without. Although the relation of compression oscillations to the arterial pressures has been repeatedly investigated there still seems to be little unanimity of opinion in regard to a number of aspects of the subject. Consider, for instance, the views which have been and are still held in regard to the interpretation of the oscillatory criterion of the diastolic pressure. Thus upon the basis of carefully conducted experiments it is held by some (4, 5) that the

¹ Read before the Washington University Medical Society October 11, 1915. Some of the results were presented at the 66th Annual session of the American Medical Association, June 23, 1915.

diastolic pressure is indicated by the highest of the oscillations obtained, while others (6, 7), upon the basis of equally careful experiments, hold that highest oscillations are recorded under a compression that exactly equals the mean arterial pressure. Again, it is maintained that the last (compressing pressure falling) of the maximum oscillations and, when that is not sharply indicated, the first sudden diminution in amplitude (3, 8, 9) indicates the diastolic pressure.² It is scarcely conceivable that these differences, involving as they do comparatively simple relations and simple methods of experimentation, could be due entirely to erroneous observation. It seemed much more likely that under certain conditions all may be right, though no one has as yet attempted to reconcile the discrepancies.

EXPERIMENTS ON ARTERIES IN A CIRCULATION SCHEMA

Methods

We had had in mind for some time an attempt to determine where the difficulty lay when a simple method of undertaking the investigation became available through the description of a procedure suitable for the purpose by Brooks and Luckhardt (12). This procedure can

² MacWilliam and Melvin (9) regard the level of external pressure just after the abrupt diminution in amplitude has taken place as the correct guide to the diastolic pressure. There is, however, less difference between the criterion adopted by them and by the author than the former seem willing to admit. I have always in my readings of the diastolic pressure looked for the sudden diminution in amplitude. Thus I say: "The moment the pressure falls below the intravascular minimum, the amplitude of the pulsation, as a rule, diminishes abruptly. In such instances it is the *last* maximum series that is obtained at minimum intravascular pressure" (3, p. 68). Again: "As the pressure continues to fall the amplitude of the oscillations of the lever will continue to increase until the pressure on the arm falls below the intra-arterial minimum. At this moment the amplitude will diminish more or less abruptly. The pressure indicated by the manometer at this moment is equal to the minimum pressure" (3, p. 67). And again: ". . . the diastolic pressure corresponds with the abrupt diminution in amplitude" (10). The fact that the diastolic pressures given in my first publication on this subject are high is not to be attributed to the use of the criterion that MacWilliam and Melvin seem to suppose we used, but rather to the employment, at that stage of our work, of a narrow arm band. The values for the diastolic pressure which these authors quote from Howell (11) and which to their apparent surprise agree so closely with those they have obtained by their *new* criterion, were obtained in routine class experiments in which the broad arm band was used, under my direction. They have not been published elsewhere.

best be made clear by describing the essentials of the simple apparatus (fig. 1) we have put together for the purpose of applying it.

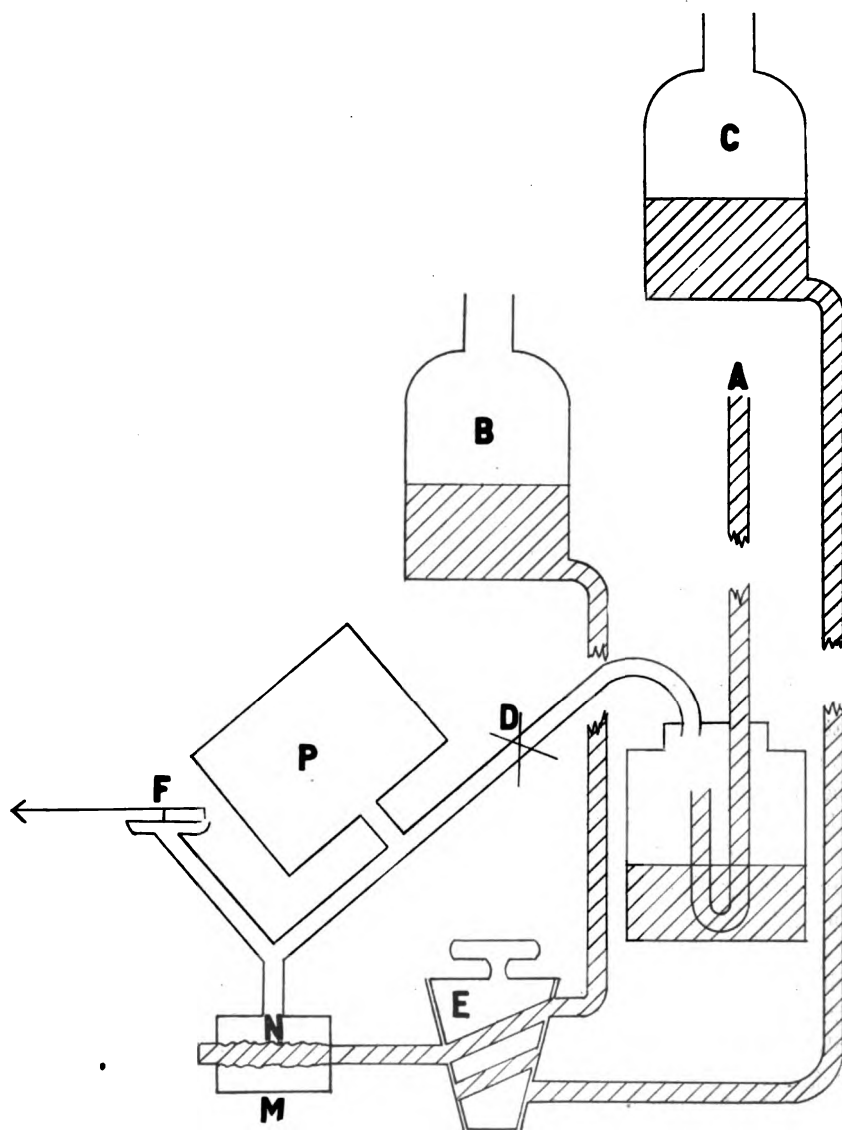


Fig. 1. Diagram of pulse schema. Description in text.

The "pulse" is produced by alternately putting the "artery," *N*, of the water-filled system into connection with one of two pressure bottles, one, *B*, determining diastolic, the other, *C*, systolic pressure. The "artery" consists either of rubber tubing about 4 mm. in diameter made of *soft*, thin rubber dam, or of the fresh carotid artery of the dog. Both are stretched somewhat, the latter to its natural length, between the cannulae by which they are held in the axis of the glass compression tube, *M*. The compression tube, measuring about 6 cm. in length and 1.1 cm. in diameter, communicates with the pressure bottle above *A*, by means of which the pressure of the air in the compression chamber can be varied; with an additional air space, *P*, whose capacity can be readily varied so as to vary the compressibility of the compression chamber as a whole, and with a recording tambour, *F*, 1.5 cm. in diameter stretched with heavy rubber dam.

The steps in the procedure consist in first turning the stopcock, *E*, so as to throw into the artery one of the arterial pressures, either diastolic or systolic, then setting the extra-arterial pressure at the desired level by adjusting the height of bottle, *A*, and closing the stopcock at *D*. Then the stopcock, *E*, is turned so as to cause the other arterial pressure, either systolic or diastolic as the case may be, to exert itself upon the artery. At each external, or compressing, pressure records are made of the level assumed by the lever of the tambour, *F*, under each of the arterial pressures in the form of short horizontal lines placed one immediately above the other, one indicating the level of the crest of the oscillation, the other that of the trough. When this is done at compressing pressures ranging from below diastolic to above systolic, a series of couples of parallel lines is obtained from which curves can be constructed so as to show the pressure oscillations transmitted from the artery to the chamber under every grade of compression.

Discussion of the method. This method of investigating the principles underlying the oscillatory criteria has certain advantages over that used by MacWilliam and Melvin and by myself:

1. In the first place it eliminates any modifying influences that the time factors of the pulse may exert upon the oscillations. This difference is considerably to the advantage of the present method, for it is obvious that the possibilities of variation in the configuration of the arterial pulse render it difficult if not impossible to take into account all of the inertia effects due to them. The Brooks-Luckhardt method, however, by eliminating the time factor provides us with the pure effects of the fundamental factors of the compression oscillations; to these

it may be possible to add by inference such disturbing inertia and resistance effects as may result from differences in the duration of the several phases of the pulse.

2. Another difference consists in the absence of flow; the resistance in our schema is infinite. This again eliminates some of the difficulties in the interpretation of results which have confronted workers in this field (3, 9). To illustrate the significance of this factor we may consider first the conditions obtaining when there is no peripheral resistance. The compression chamber itself then determines the peripheral resistance. As the compressing pressure falls the central systolic pressure will first fall and then, when diastolic compression is reached, the central diastolic will also fall. Under conditions that obtain in actual practice, however, not alone does the peripheral resistance offered by the arterioles and veins increase relatively as more and more blood succeeds in forcing its way through the compression chamber, but in addition the absolute peripheral resistance itself is unknown. It is obvious that under such conditions an exact analysis of the results obtained is scarcely possible. Standard conditions can evidently be obtained only by making the resistance infinite as we have done in this series of observations. But not alone does peripheral resistance alter the central arterial pressures in the sense mentioned above—it also will influence the rate with which the blood flows into and out of the artery in the compression chamber: a high pressure (resistance) peripherally hastening the filling and delaying the emptying of that part of the artery. The Brooks-Luckhardt method, by eliminating the time factor eliminates most of the ambiguities attributable to these effects of peripheral resistance.

3. Another advantage possessed by this method consists in the exactness with which the arterial pressures, systolic and diastolic, can be measured: it is merely necessary to measure the height of the pressure bottles. The determination of the maximum pressure of a pulse is by no means a simple matter. A valved mercury manometer accomplishes this, we believe, only approximately; the least irregularity of the pulse, even though only occasional, causes the manometer to rest at a level that does not correspond with the general levels of the arterial pressures. Furthermore, it is not often while recording the maximum pressure that we can wait until the valved manometer ceases altogether to rise, although the error resulting from measuring the distance between the base line and the line at which the maximum manometer *tends* no longer to rise probably never is very great.

GENERAL STATEMENT OF RESULTS

The results we have obtained with this apparatus show that all of the divergent views that have been held with regard to the relation to the critical compression oscillations to the arterial pressures may under certain conditions be correct. Just at which extra-arterial pressure maximum oscillations will occur, or when, in relation to the arterial pressures, sudden changes in amplitude will appear depends mainly (1) upon the compressibility of the compression space, (2) upon the extensibility of the "artery," (3) upon the ratio of upper and lower conical closures to complete closure and, especially, when compressibility of the compression space is sufficiently small, (4) upon the phase of the pulse cycle in which the compressing pressure is brought to bear upon the artery.³

THEORETIC CONSIDERATIONS

The analysis of the theory of compression oscillations that led us to believe that these are the factors that determine the form of the oscillation record, and that furnished the incentive to this phase of our investigation may well precede the consideration of our results.

1. *Incompressible transmitting medium*

a. *Inextensible artery.* The simplest set of conditions may be considered first. The compression chamber is rigid and filled with incompressible fluid; the indicator of the compression oscillations is moved by a minimal translocation of fluid; the artery is inextensible; as it collapses there is no tendency for it to stretch between the supporting cannulae; nor does it present a conical closure to the pulse. Then, the circulatory conditions remaining constant, the compression oscillations due to the pulse will depend only upon the external pressure and the phase of the pulse cycle in which this pressure is applied to the artery. The results obtained under these ideal conditions may be

³ MacWilliam and Melvin note (9, p. 165) that "the effects of external pressure upon the internal pressure are influenced to some extent according as to whether the air in the compression tube is, or is not in continuity with the air in the . . . rubber bag used for raising the pressure When the reservoir of air in the compressor is shut off there are notable differences in effect according as to whether it is shut off during the systolic or the diastolic phase." They, however, fail to note whether there were any coincident effects upon the compression oscillations obtained from the artery.

made clear by referring them to a system of coordinates (fig. 2). If the abscissae are made to represent the compressions applied to the artery and the ordinates the variations in the compression pressure effected by the pulse, the line along which the pressure in the com-

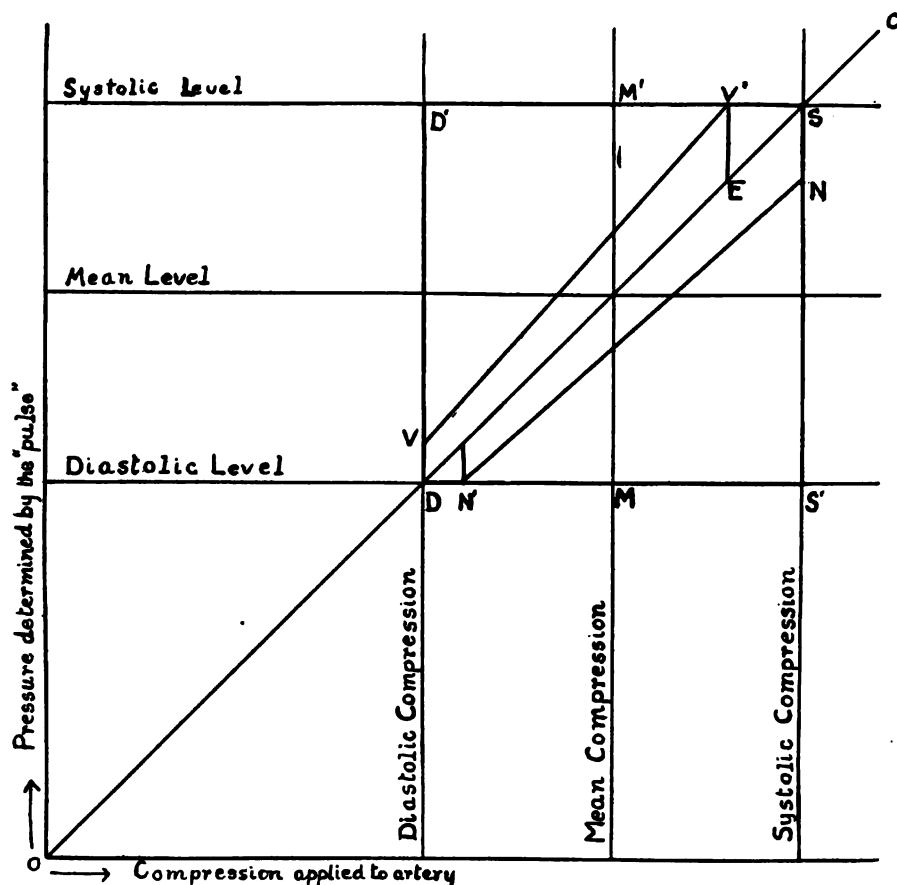


Fig. 2. Diagrammatic representation of the theory of compression oscillations.

pression chamber would rise, in the absence of pulsations, would be represented by *OC*. Now let the compressing pressure be applied in each observation while the pulse of the amplitude subtended by the "systolic-diastolic levels" is in its diastolic phase. Then during the rise of compressing pressure from *O* to the "diastolic level" the com-

pression oscillations will be nil, for during this period the artery remains full and its inextensible wall prevents any change in its volume with the pulse. At compressions exceeding the diastolic level the artery will be collapsed by the application of the pressure from without, but the pulse can now transmit its pressure through it to the compression chamber; therefore, from this level, and until the compressing pressure attains the systolic level, each pulse will raise the pressure in the compression space from its gradient, DS , to the systolic level, $D'S$. This will continue to occur until the compressing pressure, OC , exceeds the "systolic level," $D'S$, when there will no longer be in the compression space any pressure variations due to the pulse. Under these circumstances, therefore, the complete record of the compression oscillations will have the form of the triangle, $D D'S$.

If, on the other hand, the compressing pressure be applied each time during the systolic phase of the pulse the complete record of the oscillations obviously will have the form $D S'S$. It is thus seen that in one case maximum oscillations are obtained when the compressing pressure equals the diastolic pressure, in the other, when it equals the systolic pressure; and that (compressing pressure rising) in one case the diastolic pressure is indicated by an abrupt increase in the amplitude of the oscillations, in the other by the appearance of oscillations which then gradually increase in amplitude.

Again, if the compressing pressure be applied while the arterial pressure is at its mean level, the oscillation record would have the form of the figure $D M M'S$: maximum oscillations are recorded when the compressing pressure equals the mean arterial pressure.

b. *Extensible artery*: Making the artery elastic, the other conditions remaining as before, modifies the results by permitting the transmission to the compression chamber of some of the pulse pressure while the compressing pressure is below the diastolic level. And when, in addition, the attachment of the artery to the cannulae in the compression chamber results in its being stretched when compressed, the middle parts closing first, the lateral parts only after the application of considerably higher compressing pressures, not alone is this effect still further increased, but in addition pulsation is still transmitted to the compression chamber even when the compressing pressure exceeds the systolic pressure.

2. *Compressible transmitting medium*

Nevertheless, as long as the compressibility of the compression space is limited, extensibility of the artery plays a relatively unimportant part. But as the compressibility of the compression space increases, the elasticity of the artery becomes a more and more important factor, since then the range of the pressure oscillations in the compression chamber is relatively slight and the arterial walls rather than the compressing pressure therefore support the pressure exerted from within the artery.

a. *Inextensible artery.* In discussing the influence on compression oscillations of a compression space of high compressibility it is, however, convenient to again consider first the case of a wholly inextensible artery. The air-filled compression space, we will first premise, is so large that reducing it by an amount equal to the volume of the artery increases its pressure by a relatively small fraction, say $D V$ (fig. 2), of the arterial pulse pressure, $D D'$. It is also convenient to assign a relative value to the pulse pressure: let it, for the sake of simplicity, be equal to the diastolic pressure; and let the compressing pressure for the present be applied each time while the pulse is in its diastolic phase.

Upon consulting the diagram, it will be seen that with each pulse the artery under these conditions will open practically through its full diameter from the moment the compressing pressure exceeds the diastolic and until, as a compressing pressure indicated by E , the filling of the artery raises the pressure in the compression chamber to the systolic level, V' . Inasmuch as the volume of incompressible fluid entering the artery is practically the same throughout the diastolic-systolic range of compression and since at this time, as premised above, the compressing pressure is nearly twice that which obtained at D , the pressure in the compression chamber will be raised almost twice as high by the pulse at E as at D ; for the rise of pressure determined by the addition of a given volume of incompressible material to a confined, gas-filled space is proportional to the pressure of the gas filling the space. But when the compressing pressure rises above E , the increase in the volume of the artery produced by the pulse is stopped by the equal counterbalancing compressing pressure that is developed, and the pressure oscillations then diminish in amplitude in the form of the figure $E V'S$. The configuration of the oscillation record is then $D V V'S D$; maximal oscillations are recorded close to, if not at, systolic compressing pressure.

The configuration of the compression oscillations obtained when, under the same conditions, the compressing pressure is applied during systole, is represented by $S N N' D S$; for then with each pulse the artery ranges between its full, systolic size and complete collapse, excepting where at low pressures the decrease in the size of the artery lowers the compressing pressure to the "diastolic level." Under these conditions, therefore, maximal oscillations are recorded exactly at systolic compression.

b. *Extensible artery.* If now the inextensible tube be replaced by an extensible tube and one whose extensibility is the same at all pressures within the range here employed, the "full" size of the artery will no longer always be the same but will vary with the difference between the compressing and the systolic pressures; that is to say, the artery will be larger at diastolic compression than at systolic compression. If this were the only factor, it is obvious that there would be a tendency, more decided when the compression is applied during the diastolic than the systolic phase of the pulse, for oscillations to be maximal when the compression equals the diastolic pressure. This effect would add itself to the effects depicted in the preceding paragraphs, produced by the filling of the tube up to its full, but undistended state. With a tube of sufficiently small undistended bore and of sufficient extensibility the oscillations at $D V$ might well exceed in amplitude the oscillations at $E V'$. This tendency would be interfered with, though not necessarily counteracted, if the tube, like relaxed artery, were more distensible at low than at high internal pressures; for at low compressing pressures the distension attained would be then greater relatively than at high compressing pressures.

c. *Influence of the conical closures.* Finally, in order to match the conditions under which the artery oscillates in a compression chamber it is necessary to consider the influence the upper and lower conical ends of the compressed artery exert upon the oscillations. We will consider only the case of a large compression chamber. The deformation of the artery (and tissues) necessary to bring the walls of the artery together uses up some of the pressure exerted by the chamber (8). At the same time, owing to the attachment of the tube to the cannulae in the chamber, the chamber does not at its edges transmit to the artery the full pressure of its contents.⁴ Consequently the

⁴ Under natural conditions the resistance of the tissues and the way in which the arm bag transmits its pressure to the arm bring about a similar state of affairs.

ends of the compressed artery close gradually as indicated by the light lines in figure 3.

The length of the upper conical closure will vary somewhat with the arterial pressure and with the bore of the artery. Let us say that at a compression exceeding the systolic arterial pressure the apex of the cone stands at *a* (fig. 3) during diastole but descends to *b* during systole. It is obvious that as the compressing pressure diminishes both *a* and *b* will move down the artery and the distance between them will increase. Let us assume that at a compressing pressure just exceeding the arterial systolic pressure, the apex of the cone oscillates with the pulse between *a'* and *b'*. Now, when the compressing pressure falls just below the systolic level, practically the whole of the anacrotic limb of the pulse will be expended in pushing the apex of the cone from *a'* to *b'*; the very crest of the pulse will, however, act to open the rest of the compressed artery to its full but unstretched bore,

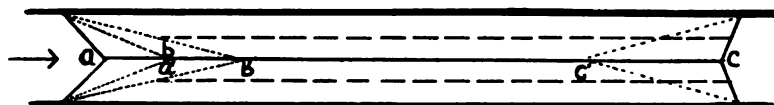


Fig. 3. Schematic representation of the movements of the walls of an artery under different compressing pressures. Heavy lines, systolic limits; light lines, compressed limits; broken lines, undistended bore; dotted lines, position of walls at upper and lower ends of compressed segment.

as shown in the figure by the broken lines, provided the time during which the arterial pressure exceeds the compressing pressure is sufficient to permit of the entrance of all of the blood needed to effect this change in volume. With further decrease of the compressing pressure the downward motion of the diastolic position of the apex of the cone continues, while the position reached by the cone when the artery opens remains fixed at *b'*, for this is the place where the artery closes at the moment the compressing pressure just exceeds the pressure in the artery, it matters not in what phase of the pulse cycle that balance of pressure develops.

The length of the lower conical closure depends upon the pressure in the artery below. It will begin to elongate upward when sufficient blood passes through the compressed artery to cause the pressure below to rise. When the artery beyond is not occluded, but possesses a peripheral resistance, this would become an important factor only toward the close of the systolic-diastolic period. The lower cone would practically cease to be a factor below diastolic compression.

Summary of the volume changes in an artery compressed in a large compression chamber, and their relation to compression oscillations

It has been deduced that pressure oscillations in a compression chamber containing a relatively large volume of air are dependent primarily upon the volume oscillations in the part of the artery compressed. Now the volume oscillations in that part of the artery are known if we know (a) the volume of the artery during diastole and (b) the increase in volume during systole. We will therefore consider the factors that have been mentioned above, together with certain additional ones, in relation to their effect (a) upon the diastolic or basal volume and (b) upon the systolic volume of the compressed artery. The factors are enumerated in tabular form (Table I) in the order in which they become effective while the pressure upon the artery is steadily decreasing.

It should be repeated here that the pressure changes in the compression chamber determined by these volume oscillations will be proportional to the pressure in the compression chamber. Therefore, throughout the entire range of falling compression there will be, in addition to all the factors mentioned in the table, a tendency for the pressure oscillations to steadily diminish in amplitude. If therefore, with falling compression, an increase in volume determined by the pulse is to cause an increase in the associated pressure oscillation, the volume change must be more than sufficient to offset the diminished effectiveness of the volume change on the pressure.

The volume tendencies given in the table have been fitted into a diagram (fig. 4, described in legend) which shows the volume oscillations thus theoretically derived. The diagram, it will be noted, presents a striking resemblance to the oscillation record usually obtained in blood pressure observations. But we hasten to add that, as respects steepness, the gradients of this diagram have been chosen arbitrarily, though in keeping with the theoretical considerations, for the very purpose of determining whether it would be possible to so derive such a resemblance. It is obvious, however, that the gradients might so vary with respect to each other as to produce very decided variations in the general configuration of the oscillation record. For instance, if the volume gradients, upper and lower, in the systolic-diastolic phase of decompression did not diverge sufficiently, the oscillations of the volume might actually produce pressure oscillations that decreased in amplitude. Such a diminution in divergence of the gradients might

TABLE I

Giving the factors determining volume changes in an artery in a compression chamber during decompression

COMPRESSING PRESSURE	DIASTOLIC VOLUME	SYSTOLIC VOLUME	VOLUME OF BLOOD MOVED BY PULSE (DIFFERENCE BETWEEN DIASTOLIC AND SYSTOLIC VOLUMES)
1. Falling to systolic level.	Diastolic position of apex of upper cone shifts from <i>a</i> to <i>a'</i> , gradually increasing the volume of blood.	Systolic position of apex of cone shifts from <i>b</i> to <i>b'</i> , increasing the systolic volume more rapidly than the diastolic volume.	Gradually increases.
2. At systolic level.	Same as at close of 1.	Increases momentarily† as far toward undistended bore of artery as time permits.	Suddenly increases.
3. From systolic to diastolic level.	a). Apex of upper cone continues to move toward <i>b'</i> , increasing volume of blood. b). Toward close of this period apex of lower cone begins to move upward, increasing volume of blood.*	a). Increases, but at a decreasing rate, through stretching of the arterial wall, and possibly through the increase in the time† the artery is open. b). As under a) above.	a). Increases rapidly at first, then more and more slowly (see below). b). There is a tendency, which may or may not be sufficient to overcome 3a) above, for the volume to decrease.
4. At diastolic level.	Marked increase as artery fills to undistended bore.	Same as at end of 3 b).	Suddenly diminishes.
5. Falling from diastolic level.	Increases steadily, but at decreasing rate, through gradual distension of artery.	Continues to increase as under 3, but the rate of increase, owing to higher pressure, is slower than diastolic increase.	Diminishes but at a decreasing rate.

* At this time also the elastic resistance of the artery to compression may begin to counterbalance a part of the compressing pressure, with the result that a certain amount of opening of the artery may occur during diastole where the walls happen to be sharply creased, thus causing a further increase in the volume of the blood.

† When the pulse has the configuration of the arterial pulse.

be due either to an increase in the steepness of the diastolic gradient, or to a decreased steepness of the systolic gradient. In a relatively wide, or short, artery, for instance, the steepness of the lower gradient might be increased through the increased importance assumed under such conditions by the upper conical closure. By increasing the length of the artery, however, the upper gradient might possibly be increased

to such an extent as to completely submerge this effect of the conical closure. Again, the upper gradient presumably would be less steep in the case of an inelastic artery. Indeed, we have shown in another place (3) that the oscillations obtained from an inextensible tube do not increase in amplitude nearly so rapidly in the systolic-diastolic range of compression as do those derived from an inextensible tube. And again, should the gradients in the earlier part of the systolic-diastolic phase of decompression be separating at such a rate as

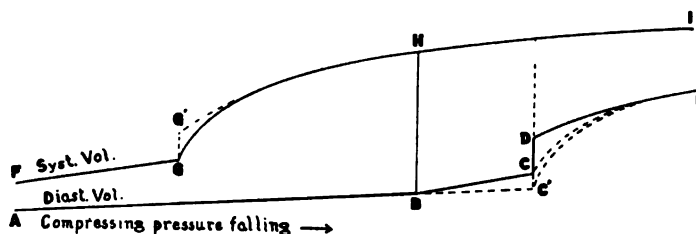


Fig. 4. Diagram illustrating the inferred volume changes of an artery in a compression chamber. The main factors concerned in determining the gradients are as follows: $a-c'$, motion downward of diastolic position of upper cone; $b-c$, motion upward of diastolic position of lower cone; $d-e$, position of the arterial wall during diastole determined by the balance between (1) arterial diastolic pressure, and (2) the compressing pressure and elastic resistance of the artery; $f-g$, motion downward of systolic position of upper cone; g or $g'-i$, position of arterial wall determined by balance between (1) systolic pressure and (2) decompressing pressure and elastic resistance of the arteries.

The amplitude of the volume oscillations with the pulse at any compression is determined by the distance between the diastolic and systolic volumes at that time. The maximal oscillation is at $b-h$.

to cause only a slight increase in the amplitude of the oscillations, the increase in the diastolic gradient due to the motion upward of the lower conical closure might cause the compression oscillations to decrease in amplitude before diastolic compression is reached. Further citations of conditions which might alter the form of the compression record are probably unnecessary, for it should be possible now to determine their effects by fitting them into the accompanying table and figure.

EXPERIMENTAL

Oscillation diagram. In none of our experiments with the circulation schema have the conditions corresponded exactly with any one of the sets discussed above. (1) Thus we have not attempted to com-

pletely eliminate compressibility of the compression space, but rather have experimented with compression chambers of relatively high and relatively low compressibilities. (2) In our experiments closure of the artery could occur only with a certain amount of stretching between the cannulae supporting it in the compression chamber. This factor was relatively greater with rubber tube than with artery, because of the greater bore of the former. (3) By reason of the attachment of the artery to these cannulae the effects of the upper conical closure of the *artery* are probably more important relatively than in estimations made under natural conditions. In this connection it should again be borne in mind that our rubber tubes were wider than the arteries; consequently under the same conditions the effects of the upper conical closure should be more striking, in the case of the former; in effect, the compression chamber, although of constant length, was *relatively* longer when it contained artery than when it contained rubber tube. (4) Neither have any experiments been made, owing to practical difficulties, with wholly inextensible, though flexible tubing. Nevertheless, the conditions have been varied sufficiently to render the results of value as a check on the foregoing theoretical considerations.

Here it might be mentioned that a pressure of about 1 to 2 cm. of water was required to plumply fill the rubber tube used in these experiments. Beyond this point and within the range of pressure herein employed, the extensibility of the rubber tube was practically proportional to the distending pressures. Estimations of the extensibility of the artery were not made. Inasmuch as we were dealing with relaxed artery, it may be assumed (9) that its extensibility decreased as the internal pressure increased. It might also be mentioned here that a certain amount of pressure was necessary to just bring the middle segment of the walls into apposition with each other. With rubber tube this amounted to 3 to 4 cm. of water. On account of its much smaller bore it was rather difficult to determine the pressure necessary to just collapse the artery. It may, however, be assumed that it was approximately the same as in the case of the rubber tube.

With our apparatus it was convenient to begin each set of observations with low extra-arterial pressure and to increase this pressure in steps from below diastolic to above systolic pressure. The results have been plotted in two ways. (a) In order to make clear the general configuration of the records we have in a few instances plotted the oscillation pressures (ordinates) against the initial compressing pressures (abscissae). And (b) in order to facilitate a comparison of the present

records with the records as usually obtained in blood pressure observations, we have in all cases plotted the *amplitude* of the oscillations against the initial compressing pressures. It should be added here that

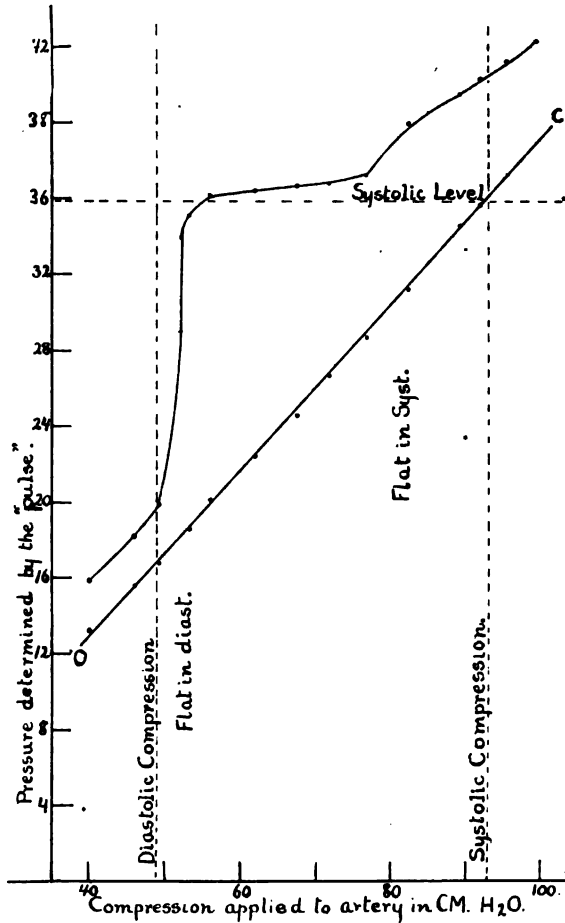


Fig. 5. Curve reconstructed from a record of the pressure changes in a compression chamber. Rubber tube; small compression space closed during diastole. The word "flat" in figures 5 to 16 when unqualified, is intended to mean that only the central part of the artery is just collapsed.

the deflections of the manometer connected with the compression chamber, which was calibrated in each experiment, were equal for equal increments of pressure within the range employed.

General configuration of records

Small compression chamber. The compression chamber made small by the means described above had an air space of about 10 cm. The configurations of the plots (figs. 5, 6, 7) made from experiments in which the rubber tube served as "artery" in this chamber bear a strik-

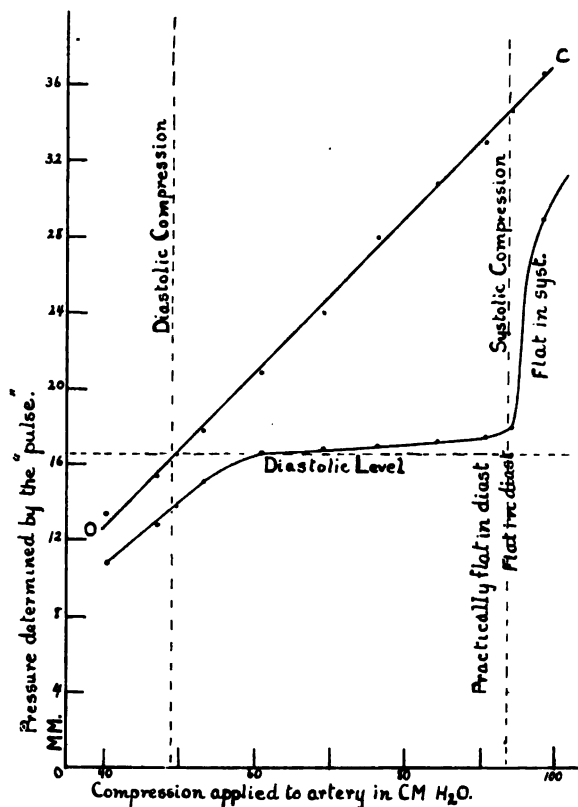


Fig. 6. Curve reconstructed from a record of the pressure changes in a compression chamber. Rubber tube; small compression space closed during systole.

ing resemblance to the diagram (fig. 2) constructed from theory. Thus when the compression is applied each time while the pulse is in its diastolic phase (fig. 5) the oscillations are built up on the gradient, OC , of the compressing pressure and have a configuration tending decidedly toward $D D'S$ of figure 2. Again, when the compression is applied each time while the pulse is in its systolic phase (fig. 6), the oscillations

are built down from the gradient, OC , of the compressing pressure and have a configuration tending decidedly toward $D S' S$ of figure 2. And finally, figure 7, depicting the results obtained when the compression is applied while mean arterial pressure prevails, simulates in configuration $D M M' S$ of figure 2.

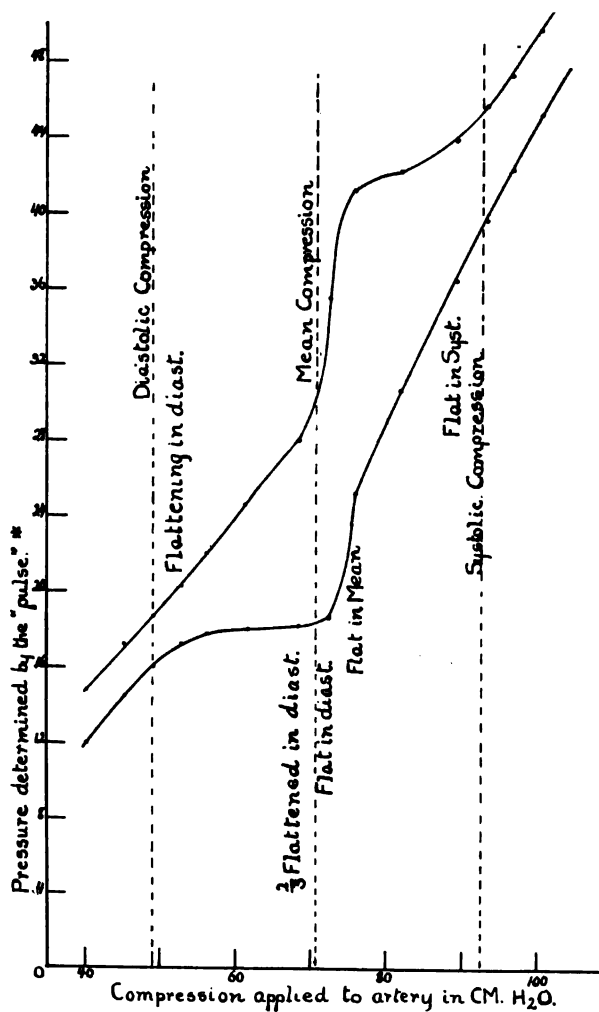


Fig. 7. Curve reconstructed from a record of the pressure changes in a compression chamber. Rubber tube; small compression space closed at mean arterial pressure. Only the relative ordinates of this curve are known.

We have not deemed it necessary to reproduce similar reconstructions of the records obtained from artery. Inspection of the reconstructions showing the oscillation amplitudes obtained in the case of artery (figs. 8 and 9) is all that is now needed to render obvious the general configurations of those records. The variations from the configuration of the curves obtained with rubber tube thus brought out are attributable to the fact that the volume of the artery relative to that of the compression space is less than that of the rubber tube; it therefore happens that the elastic arterial wall rather than the compression chamber supports the arterial pressure while the compression pressures are low.

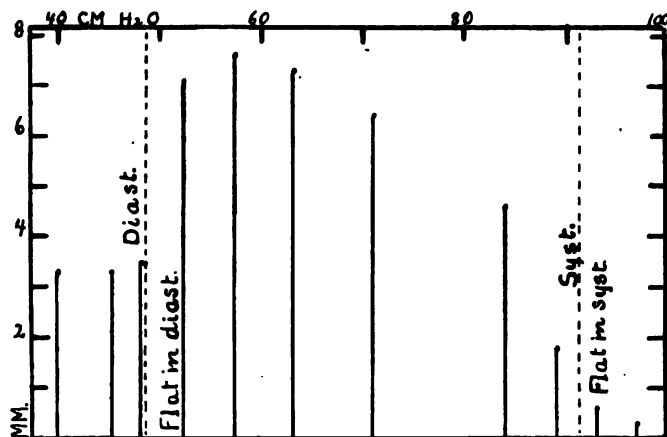


Fig. 8. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Artery; small compression space closed during diastole. In figures 8 to 16 the abscissae indicate the compression pressure in cm. of water, the ordinates the amplitude of the compression pulse in mm.

When the compression is applied during the diastolic phase of the pulse (fig. 8) the oscillations, under compressions ranging within the pulse pressure, are high at first, and more or less uniform; they then decrease, though not to so low an amplitude as at corresponding pressures in the case of rubber tube. And when the compression is applied during systole (fig. 9) the oscillations in the diastolic-systolic range at first increase in amplitude and then decrease, though not to so low an amplitude as is obtained at corresponding pressures in the preceding case.

Large compression chamber. The air space of the compression chamber was then considerably increased, but in all tests of this set the com-

pressibility was adjusted so that the oscillations, whether from rubber tube or artery, were under similar conditions of approximately the same amplitude. Figures 10 and 11 illustrate the results obtained with the rubber tube in this larger chamber. In accordance with theory, it is seen that the amplitude of the oscillations increases slightly and more or less consistently through the diastolic-systolic range of compression. The results differ slightly, though in keeping with theory, according as the compression is applied during the diastolic (fig. 10) or the systolic (fig. 11) phases of the pulse.

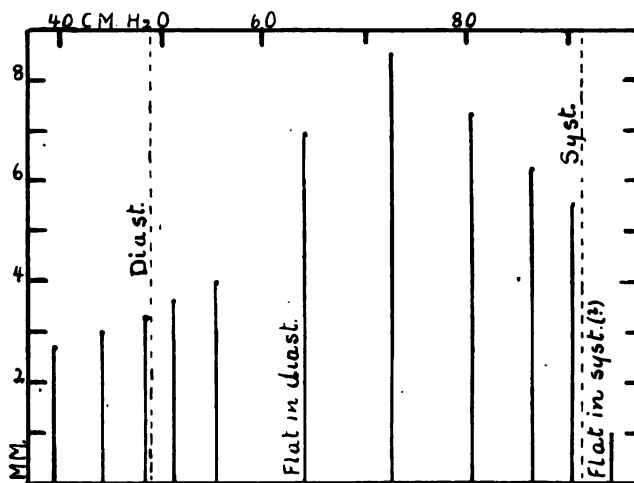


Fig. 9. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Artery; small compression space closed during systole.

With artery, however, it is seen (figs. 12 and 13) that in the same range of compression the amplitude of the oscillations on the whole *decreases* in both cases, though again there are slight differences depending upon the phase of the pulse in which the compression is applied.

At first thought it might seem that this result is just the reverse of the one the theoretical considerations should lead us to anticipate; for the extensibility of artery is such as to have the effect of enhancing the decrease in the amplitude of the oscillations as seen in the diastolic-systolic range in the case of rubber tube. When, however, it is recalled that the bore of the artery is much smaller than that of the rubber tube, and that this has the effect of making the compression chamber longer

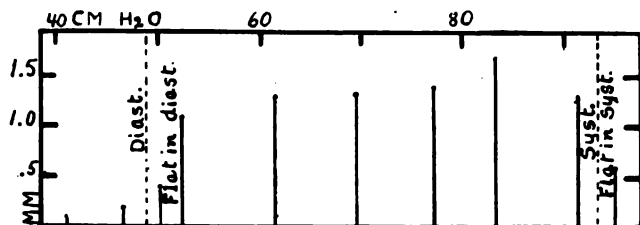


Fig. 10. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Rubber tube; large compression space closed during diastole.

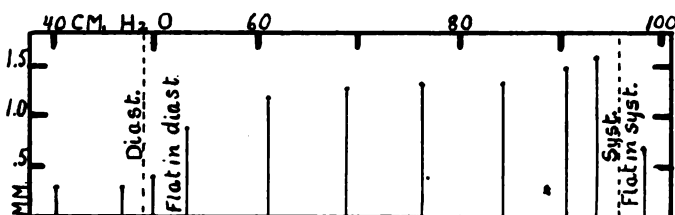


Fig. 11. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Rubber tube; large compression space closed during systole.

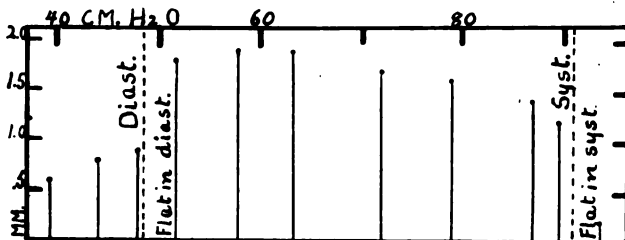


Fig. 12. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Artery; large compression space closed during diastole.

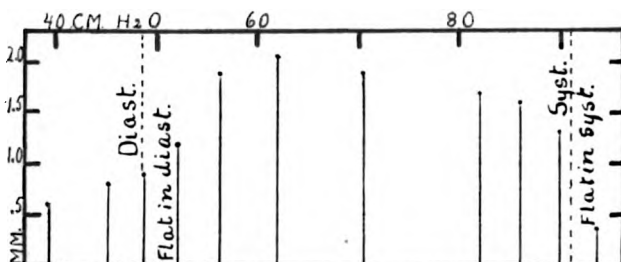


Fig. 13. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Artery; large compression space closed during systole.

relatively in the case of the artery, a satisfactory explanation of the results obtained with artery is found: the effect upon volume exerted through the upper conical closure is submerged by the effect upon volume exerted through the influence of the long bore of the artery (see below); though it is possible that in part this result is attributable also to an assumed smaller undistended bore and greater extensibility of the artery relative to the rubber tube.

Effect of length of artery upon the configuration of the oscillation record

In order to test the theoretical considerations that have to do with the influence of the length of the tube upon the configuration of the oscillation record, the oscillations obtained from a long (10.5 cm.) rubber tube have been compared with those from a short (5.4 cm.) rubber tube, under conditions as nearly alike as they could be made, excepting that the compressibility of the chamber was regulated so that the compression oscillations were of approximately the same height in both tests. These tests fully confirm the premises. With a longer tube the amplitude of oscillations within the diastolic-systolic range of compression changes very little; and the diminution in amplitude when the systolic level is reached is quite abrupt. With the shorter tube the results resemble closely those illustrated by figure 10: the oscillations increase in amplitude to the systolic level, and then decrease, though not so abruptly, as in the case of the longer tubes.

Critical oscillations

Turning now to the relation of the oscillation amplitudes and amplitude changes to the diastolic, mean and systolic arterial pressures, it is well first to recall that a pressure of from 3 to 4 cm. of water is necessary to bring the walls of the tubes into apposition with each other, and therefore that such compressing pressures as collapse the artery must be corrected by deducting 3 to 4 cm. from them.

Small compression chamber. Bearing this in mind and making allowance for the gaps between successive readings, it is seen (figs. 8 and 14) that when the compressing pressure is applied while diastolic pressure prevails the amplitude of the oscillations increases abruptly the instant the extra-arterial pressure exceeds the intra-arterial diastolic pressure, and that this increase is practically to the maximum. On the other hand, the decrease to the small oscillations that obtain when

the intra-arterial systolic pressure is exceeded takes place more or less gradually.

When the compression is applied while systolic pressure prevails in the system (figs. 9 and 15) the diastolic pressure, instead of being marked by an abrupt increase in oscillation amplitude, is indicated either by an amplitude of oscillation which begins to increase from a constant height (rubber tube) or which increases at a more rapid rate (artery); while the systolic pressure now is marked by a decrease in amplitude that is decidedly abrupt. Maximum oscillations are obtained either at systolic pressure (rubber tube) or considerably below systolic pressure (artery).

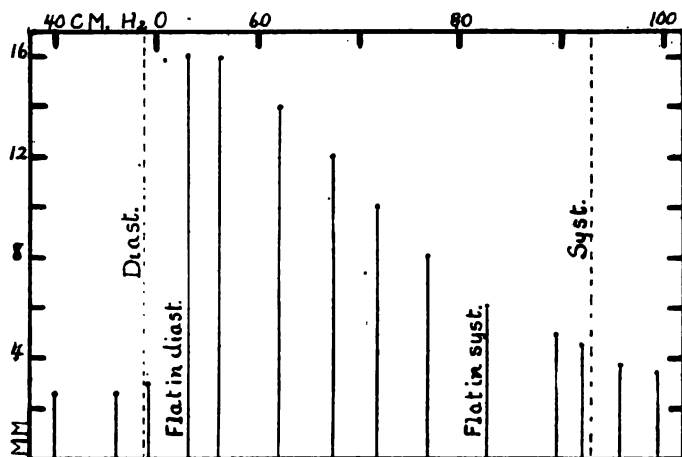


Fig. 14. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Rubber tube; small compression space closed during diastole.

When the compression is applied while mean arterial pressure prevails⁵ the diastolic pressure (fig. 16) is indicated by a gradual increase in the amplitude of oscillations, systolic by a decrease in the rate of diminution; while maximal oscillations are obtained approximately at mean compression.

Large compression chamber. With the larger compression chamber, however (figs. 10, 11, 12 and 13), both the systolic and the diastolic pressures are invariably indicated by abrupt changes in the amplitude of the oscillations. The increase in the amplitude of the oscillations

⁵ Done with rubber tube only.

at diastolic pressure is, with both rubber tube and artery, more abrupt when the compression is applied during diastole (figs. 10 and 12) than during systole (figs. 11 and 13); while under all circumstances it is more abrupt with artery than with rubber tube. Indeed, almost within the limit of error of measurement, the increase in amplitude when the compression is applied to artery during diastole is to maximum at once. And under all conditions, systolic pressure is marked by an abrupt decrease in the amplitude of oscillations. In accordance with theory the decrease is more marked with the rubber tube than with artery and when the compression is applied during systole than during diastole.

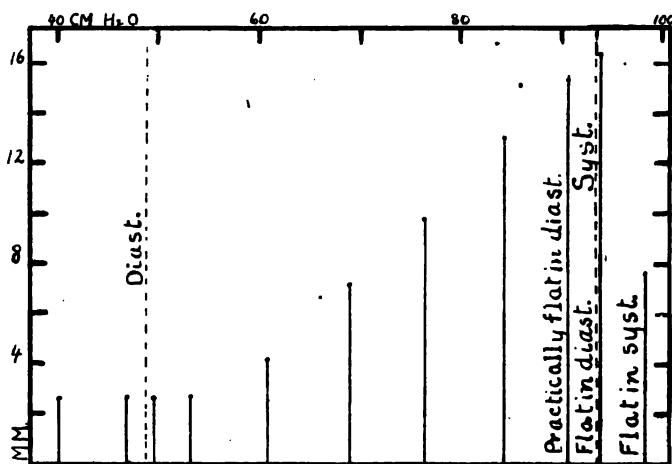


Fig. 15. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Rubber tube; small compression space closed during systole.

The influence on the oscillations of reducing the duration of the "pulse." A study of the influence on the oscillations of reducing the duration of the "pulse" has not been included in the present investigation. In our first investigation of this subject (3), however, a schema was used in which the pulses recurred at the rate of about 60 per minute. The records obtained at that time from rubber tubes made in the same way as were those employed in the present research, show that the oscillations in the systolic-diastolic range have a decided crescendo up to diastolic compression. In view of the fact that under the slow "pulse" of the present investigation the oscillation amplitude in the same range tended to decrease rather than increase, we feel justified

in concluding that the differences are attributable to the differences in the duration of the "pulses." This result, as a matter of fact, might have been predicted, for if time is a factor it must become increasingly potent as the compression decreases from the systolic to the diastolic level, since the time during which the effective pressure lasts increases throughout this period from a mere moment, under systolic compression, to the duration of the pulse itself under diastolic compression.

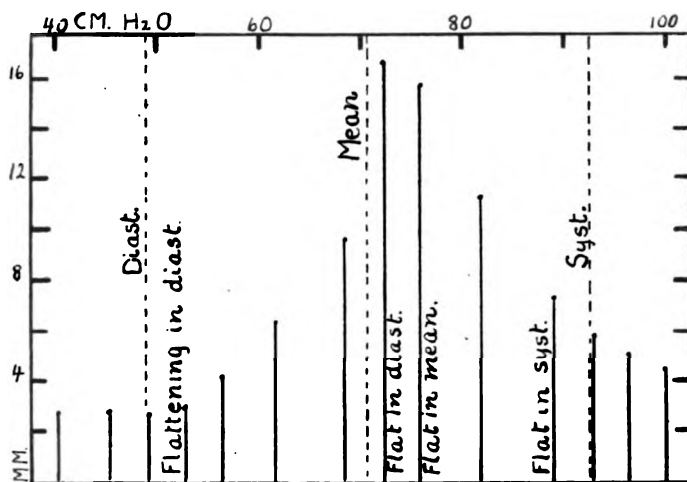


Fig. 16. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Rubber tube; small compression space closed during mean compression.

Discussion of discrepancies in the literature

The marked influence exerted by the size of the compression space upon the relations obtaining between oscillation magnitude and arterial pressures in large measure accounts, we believe, for the diverse conclusions reached by different workers in this field. An attempt to account for all the differences recorded in the literature, in the light of our results, would however scarcely be worth while even if the articles contained the necessary data, and usually they do not. We do, however, desire to say a word with regard to the experiments of Mac-William and Melvin (9), since these investigators, using a circulation schema similar to the one originally used by the author, have reached conclusions differing in some respects from those recorded by us. Thus while we found that the last of the series of maximal oscillations were

obtained at compression pressures that agreed fairly well with the arterial diastolic pressure, MacWilliam and Melvin find no such correspondence; in their experience maximal oscillations were obtained considerably above diastolic compression, while diastolic pressure seemed to be indicated rather by a sudden change in amplitude.

It will be noted that this is exactly the result obtained by us in the present series of experiments, when artery, under most circumstances, is pulsating in a comparatively small compression space. Now, although MacWilliam and Melvin do not give the dimensions of their compression chamber, we may be justified, by the fact that they used a considerable length of *large* artery, in assuming that it was small relatively. If so, the failure of the last maximal oscillations to agree with diastolic arterial pressure noted by them undoubtedly is partly referable to the great variation in the size of the artery (carotid of sheep and of ox) they employed, and also to the way in which they varied the volume of air in their compression chamber, to be inferred from the statement that "air was commonly used as the transmitting medium."

Motion of arterial walls in relation to critical oscillations and arterial pressures

The method we have used for the purpose of studying the compression oscillations offers a splendid opportunity for observing the relation of the swing of the arterial wall to the critical compression oscillations. These observations have shown (see the notes inscribed on figs. 5 to 16) that *the* maximal oscillation is obtained under all circumstances at a compression within the range that brings the arterial walls into apposition, it matters not what the relation of the maximal oscillation to the arterial pressures may be. This observation is in striking contrast to that of MacWilliam and Melvin who state that maximal oscillations develop when normal distensible artery⁶ is compressed only to the "half-flattened" state.

⁶ MacWilliam and Melvin, however, find that with non-distensible tube or artery maximum oscillation occurs when the compressing pressure causes complete or almost complete flattening during diastole. Is it possible that their non-distensible tubes and arteries were smaller than their distensible tube and that this difference in the behavior of the two types of tube is referable rather to differences in the relative compressibility of the compression space thus determined and to differences in the amount of fluid, i.e., the time, required to fill the tubes, than to differences in extensibility of the tubes?

While MacWilliam and Melvin surmise that the discrepancy is referable to differences in the time factors of the "pulses" and possibly in the volume of "blood" moved under the compression in the two sets of experiments, they nevertheless seem to be satisfied that their results and the explanation they give to account for them are directly applicable to conditions that obtain in blood pressure observations in man. It can easily be shown, however, that this attitude is wholly untenable.

The pulse in the circulation schema used by me (3) was developed by a rotating stopcock which put the "artery" into communication with the head of pressure during one-third of each revolution. We showed that the wave thus developed resembled the arterial pulse fairly closely. MacWilliam and Melvin presumably produced their pulse by an electro-magnetic interrupter which "temporarily" occluded the tube leading from the pressure bottle usually 60 times a minute (13). These investigators

fail to give the duration of the temporary occlusion, nor do they say anything in regard to the configuration of the pulse thus obtained. An examination of their curves with a magnifying glass makes it ob-

vious, however, that they were not dealing with a pulse of the usual form but rather with a very complicated one in which the pressure was low for a very brief period and high for a comparatively long period. Now a pulse of this form accounts perfectly for the fact that in their experiments the "artery" did not flatten under a compression that exceeded the diastolic pressure.

For the purpose of making this clear we will regard the arterial wall as a membrane, *M* (fig. 17) free to move between two opposing pressures, the arterial pressure acting from one side, the compressing pressure from the other. The movements of this membrane are limited, on one side by contact with the opposite wall (that is by complete collapse) and upon the other by the distended position of the wall. Under these circumstances the membrane would be held against one wall as long as the excess of pressure was in that direction, and would move completely across the space to the other wall the instant the excess of pressure was in the other direction. This would be the type of motion

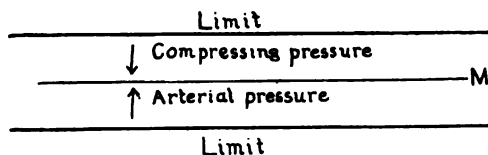


Fig. 17. Diagram to illustrate the influence of the configuration of the pulse upon the movements of the arterial wall.

as long as the compressing pressure lay within the pulsatile range of arterial pressure. But let us now assume that this membrane is so damped by the fluid around it, or, if you will, upon one side of it, and that the phases of the pulse are so brief that the disturbances of the equilibrium determined by the pulse do not last long enough to carry the membrane to these limits. Under such circumstances, which are indeed the circumstances obtaining in an artificial circulation schema and possibly also in actual estimations of the blood pressure, the position assumed by the membrane would in addition depend upon the relation of the arithmetic mean of the maximum and minimum pressures of the pulse to the geometric mean of the pulse area. If the geometric mean lies to the systolic side of the arithmetic mean, that is, if the pressure is high for a longer period than it is low, the membrane will not have time during the period of low pressure to reach the lower limiting side and hence will oscillate from the upper wall for a part of the period during which the compressing pressure lies within the range of the pulse pressure: the artery would then close no further than to the "half-flattened" position of MacWilliam and Melvin. If, on the other hand, the geometric mean lies to the diastolic side of the arithmetic mean, which is true of all arterial pulses, the membrane, within the same range of pressure, would oscillate from the lower wall of the diagram, but might not attain the upper wall. That is to say, throughout the pulse pressure range of compression the artery would be completely collapsed during a part of each pulse cycle; it would cease to resume the collapsed position at some time in each pulse only when the compressing pressure became less than the diastolic pressure.

When, in the present experiments, the compressibility of the compression space is small, to return to a discussion of our results, maximal oscillations under all circumstances (figs. 8, 9, 14, 15, and 16) coincide more or less exactly with the compression that *just* flattens the tube and if, in addition, the compression has been applied during the diastolic phase of the pulse, this occurs more or less exactly at diastolic compression (figs. 8 and 14).

When the compression space is large (figs. 10, 11, 12 and 13) the artery is *just* flattened exactly at diastolic compression under all circumstances. Reading the figures backward, last flattening is always immediately followed by a sudden diminution in the amplitude of oscillations, and when the compression is applied to *artery* during diastole (fig. 12), last flattening and *last* maximal oscillation agree exactly with diastolic compression.

EXPERIMENTS ON ARTERIES IN SITU

Methods in general

The methods employed in the present animal experiments will be fully described elsewhere. Here it will suffice to say that the compression was applied to the dog's femoral artery through our arteriograph (3). The compression chamber usually had a capacity of from 0.5 to 1 L., approximately. The pressures in it were recorded photographically by a Frank mirror capsule connected with the compression space either directly, or indirectly through the author's sphygmomanometer. The records were made both by the method of continuous escapement, when the recording tambour was provided with a pin-hole opening, and by the method of intermittent escapement, when the tambour system was completely closed while the records were in the process of making. The arterial pressure was not recorded but the sounds heard in the arteries below the compression chamber were signaled and often recorded also. The pulse peripheral to the compression chamber was also recorded by a Frank mirror capsule. The relation the sounds bear to the oscillations will form the subject of another communication; for the present it will be arbitrarily assumed that the appearance of sound indicates the systolic pressure and that the dulling of the sounds at the end of the 3d phase is an index to the diastolic pressure. The conditions obtaining in the animal experiments resemble those of a long artery in a large compression chamber.

RESULTS

General configuration of the compression records

It was not expected that these methods would shed any new light on the general configuration of the compression record as a whole. It is, however, worth while noting that the records obtained with the Frank capsule by both the continuous and intermittent escapement methods have exactly the same general outlines as those that are obtained with the usual apparatus. The records are too long for reproduction here but their contours may be judged by the measurements of the oscillation amplitudes of a number of typical records which have been collected in Tables II and III. It is there seen that the very gradual increase in amplitude at first recorded during decompression shows a decided acceleration, beginning about 5 to 15 mm. Hg. above the level at which the first sound is heard, the largest absolute increase,

TABLE II

Analysis of photographic records of blood pressure estimations by the method of continuous escapement (tambour pin hole open) from the bare artery of the dog.

dog no. 10			dog no. 13		
No. of pulse*	Oscillation amplitude	Time to peripheral pulse	No. of pulse†	Oscillation amplitude	Time to peripheral pulse
	mm.	sec.‡		mm.	sec.§
1	4.5		5	2.0	
2	6.5		9	2.0+	
3	7.5		13	2.0	
4	6.5		17	2.5	
5	10.5		21	3.0	
6	9.0		25	5.5	
7	11.0		29	5.5	
8	12.5		33	5.0	
9	11.5		37	6.5	
10	16.0	0.071	41	14.0	
11	13.5		42	17.0	
12	16.0	0.063	45	21.0	0.085
13	17.0	0.071	46	19.0	0.102
14	16.5	0.071	49	21.0	0.099
15	18.5	0.060	52	25.0	0.074
16	18.0	0.079	55	25.0	0.076
17	19.5	0.059	59	26.0	0.063
18	20.0	0.056	64	26.0	0.063
19	19.0	0.066	69	28.0	0.063
20	20.5	0.053	73	28.0	0.048
21	20.5	0.054	77	27.0	0.054
22	21.0	0.048	81	28.0+	0.05
23	22.0	0.048	85	29.5	0.046
24	21.0	0.057	89	30.0	0.04
25	22.5	0.043	93	30.0	0.038
26	22.0	0.043	97	31.0	0.038
27	22.0	0.046	101	33.0	0.043
28	23.0	0.038	105	36.0	0.037
29	22.0	0.043	109	39.0	0.031
30	23.0	0.037	113	40.5	0.036
31	23.0	0.035	114	40.0—	0.038
32	23.0	0.043	115	40.5	0.038
33	23.5	0.032	116	41.0	0.034
34	23.5	0.034	117	40.0	0.038
35	24.0	0.035	118	40.0	0.032
36	24.0	0.028	119	41.0	0.037
37	24.0	0.038	120	40.0	0.031
38	25.0	0.026	121	40.0—	0.032

TABLE II—Continued

DOG NO. 10			DOG NO. 13		
No. of pulse*	Oscillation amplitude	Time to peripheral pulse	No. of pulse†	Oscillation amplitude	Time to peripheral pulse
	mm.	sec.‡		mm.	sec.§
39	25.5	0.028	122	41.0	0.031
40	26.0	0.026	123	39.5	0.027
41	26.0	0.025	124	38.5	0.031
42	27.0	0.028	125	38.0	0.025
43	26.0	0.025	126	37.0	0.029
44	26.5	0.024	127	34.0	0.021
45	23.0	0.024	128	33.5	0.029
46	23.5	0.019	129	32.5	0.024
47	24.5	0.021	130	30.5	0.024
48	19.0	0.013	131	28.0	
49	18.5	0.012	132	30.0	0.018
50	14.0	0.013	133	27.5	0.02
51	14.5	0.010	136	25.5	0.016+
52	15.0	0.012	138	24.5	0.016
53	13.0	0.088			
54	13.0	0.088			
55	11.0	0.073			
56	11.0	0.059			
57	11.5	0.044			
58	10.0	0.029			
59	10.0	0.029			
60	9.5	0.044			
61	9.0	0.029			
62	9.0	0.029			

* The mean rate of pressure decrease is 2.2 mm. Hg. per pulse; the rate in the vicinity of systolic pressure and of diastolic pressure is approximately 2.5 + mm. Hg. and 1.9 - mm. Hg. per pulse, respectively.

† The time was recorded in seconds. The paper, however, moved very uniformly at the rate of 68 mm. per second. The figures given in this column are therefore approximately correct.

‡ The rate of fall of pressure in the vicinity of pulse No. 5 is roughly 1 mm. Hg. per pulse; in the vicinity of pulse No. 123 it has decreased to approximately 0.5 mm. Hg. per pulse.

§ The time is recorded in fifths of seconds. The speed of the paper was not absolutely uniform but varied regularly between 16 and 20 mm. per 0.2 second. 1 mm. may therefore be regarded as roughly equivalent to 0.0112 seconds.

¹ First sound.

² Sound fainter.

³ Possibly fainter.

⁴ Certainly fainter.

TABLE III

Analysis of photographic records of the blood pressure estimations by the method of intermittent escapement (tambour pin-hole closed) from the bare artery of the dog.

DOG NO. 14			DOG NO. 15			DOG NO. 19		
Com- pressing pres- sure	Oscillation amplitude	Time to peripheral pulse	Com- pressing pres- sure	Oscillation amplitude	Time to peripheral pulse	Com- pressing pres- sure	Oscillation amplitude	Time to peripheral pulse
mm. hg.	mm.	sec.	mm. hg.	mm.	sec.	mm. hg.	mm.	sec.
160	1-1.5		155	1		170	1.0	
155	1-1.5		150	1+		165	1.3	
150	1.5-2.5		145	1+		160	1.5	
145	3.0		140	2-5.5		155	2-4.5	
140	6.5-8.5		135*	6-13	0.082-0.094	150*	5-8	
135	6.5-9.0		130	10-15	0.072-0.078	145†	7-10	
130*	11-14.5	0.079	125	17-22	0.068	140	10-11	0.054
125	15-15.5	0.079	120	20-23	0.034	135	11-11.3	0.043+
120	16-18	0.055	114	22-24	0.034	130	12+	0.046
110	18-18.5	0.049-0.055	110	24-25.5	0.044	125	12.5	.036
105	17.5-18	0.049-0.051	105	26-27	0.042	120	13.0-13.5	0.034
100	18.0	0.041	100	26-27.5	0.038-0.042	115	13.5-13.7	0.027
95	19.5-20	0.031-0.035	95	27-27.5	0.038	110	14.0	0.0238
90†	16-18	0.017-0.021	85	28.5-29	0.038	105	12.5	0.0222
85	12-12.5	0.015-0.017	80†	24-25	0.022-0.028	100†	10.0	0.0155
80	8.5-9.0	0.009-0.011	75	16-20	0.028	95	8.0	0.014-
			70	16.0	0.02-0.016	90	6.0	0.0126
			60	11.5	‡	85	5.5	0.0126-0.0142
						80	5.2	0.0142

* First sound.

† Sounds fainter.

‡ Pulse first becomes visible.

§ Pulse wave not clear.

however, coinciding, within the limit of error of the method, with the very pulse that determines the first sound. Then the increase in amplitude becomes more and more gradual. In about half of the records this gradual increase either continues, or the oscillations attain and maintain a uniform height, until an abrupt diminution in amplitude is registered; in the other half a gradual though slight diminution in amplitude is seen to begin a variable time before an abrupt diminution occurs. The dulling of the sounds always coincides *exactly* with the sudden diminution in amplitude. The general configuration of the records therefore agrees perfectly with the theoretical consideration.

Volume pulse of the compressed artery

Inasmuch as the theoretical development of this subject has indicated that the volume changes of the compressed artery are probably of prime significance in the production of compression oscillations, an

attempt has been made to gain some specific knowledge with regard to the actual volume changes an artery experiences while it is being gradually decompressed.

To obtain this information the following method has been employed. The arteriograph and a horizontal glass tube 3 mm. in bore extending from it to an air chamber of about 1 liter capacity, were filled with water to the total exclusion of air to a chosen position in the tube. The meniscus of the water in the tube therefore moved to and fro as the volume of the artery changed with the pulse and with the pressure exerted back upon it by the air in the air chamber beyond. The motion of the meniscus was photographed by causing its image to fall upon the slit of a photo-registering apparatus. The tube was calibrated by measuring the distance the image of the meniscus moved when known amounts of water were allowed to flow into it. Inasmuch as the volume of water moved by the pulse in this apparatus is quite considerable the position attained by the meniscus during systole is probably subject to some error. Nevertheless, the general accuracy of the method is indicated by the fact that the bore of the artery derived by calculation from the volume change as given by the record and the known length of the artery compressed, shows a surprisingly close agreement with the actual bore of the artery. Thus in the experiment here used for purposes of illustration the maximum increase in volume from the zero level is something over 0.6325 cc. (see Table III). The effective length of the arteriograph, that is, the length of artery compressed, is something over 5 cm. Therefore the maximum bore of the artery is (Vol. = $\pi r^2 \times L$; or $r = \sqrt{\frac{.6325}{\pi \times 5}} = 0.2$ cm.) 4 mm. How nearly

correct this result is may be judged by the fact that the orifices of the arteriograph measure 5 mm. in diameter and that while adjusting this instrument it was always moved up the artery until the latter just filled the orifices without actually being pressed upon.

The beginning of the 1st phase and the beginning of the 4th phase of the Korotkoff sounds were signaled upon the same record. It should be added that for reasons to be considered in another connection the early first phase sounds may not be as distinct as usual, and may even be missed, when the compression tube is filled with water. Therefore, the first signal in these experiments probably was often later by a few pulse waves than it would otherwise have been.

We are reproducing here one of the records of the volume changes of the compressed artery (fig. 18), greatly reduced in size. The first sig-



Fig. 18. Record of the volume changes of an artery in the compression chamber while it is being gradually decomposed. Reduced to $\frac{1}{4}$ size. The record reads from right to left. On the original an elevation of 1 cm. equals a volume change of 0.055 cc., approximately.

nal, for the reason just given, is undoubtedly several pulse beats late. We will assume it should have signaled the 5th pulse of the record. The 2d signal, allowing for reaction time, signals the 38th pulse. The record is analyzed in Table IV. If the wave-like fluctuations, which probably are caused by respiratory blood pressure changes, are disregarded, the resemblance of the contours of this record to those of figure 4 is seen to be very close. Thus, the basal or diastolic volume rises very gradually, during 31 pulses, to within 6 pulses of the diastolic pressure (beginning of the 4th phase sounds). With the 32d pulse it begins to increase somewhat more rapidly, though still relatively slowly, until diastolic compression is attained (37th pulse), when a much more rapid increase in the basal volume begins—an increase which, judging by the new curve it follows, must be due to the entrance of a wholly new factor. Now, if we assume that the first gradual increase in the diastolic volume is due to the extension downward of the upper conical closure of the artery,⁷ we should expect the lower cone to add at least a like amount of blood when it begins to manifest itself. This latter addition to the volume of the blood in the compression tube should occur rather rapidly when the peripheral artery begins to fill rapidly, that is, when the compression approaches the diastolic level, and the effects at the two cones should then summate. It is therefore interesting, to say the least, that the first slow rise involves an addition of 0.0577 cc. of blood, while the total rise at diastolic compression (37th pulse) amounts to 0.1402 cc., an increase of about $2\frac{1}{2}$ volumes; the relation of the two volumes to each other is quite in keeping with the premises. And it is probably more than a mere coincidence that the volume obtaining during systole just before the pulse presumably breaks through (5th pulse—0.0825 cc.), i.e., when the upper cone is manifesting its maximum effect upon the volume of the compressed artery—is roughly one-half of the total increase in volume (0.1402 cc.) attained during diastole just before the lower conical closure is obliterated by the opening out of the whole length of the artery (37th pulse). It is, however, realized that this relation is influenced somewhat by the level of the compressing pressure at which the record happens to start and by the resistance the blood happens to experience in flowing out of the artery into the veins.

Finally it seems justifiable to conclude that the new factor that has

⁷ This is probably the most important factor; a certain amount of the increase may be attributable to the opening out of the artery at points where it is sharply creased by the compression.

TABLE IV

*Analysis of a record (fig. 18) of the volume changes in a compressed artery.
Method of continuous escapement. Dog No. 21*

NO. OF PULSES	ELEVATION AT END OF DIASTOLE	VOLUME AT END OF DIASTOLE	ELEVATION AT CREST OF PULSE	VOLUME AT CREST OF PULSE	AMPLITUDE OF OSCILLATIONS	VOLUME OSCILLATION WITH ANACROTIC LIMB
	mm.	cc.	mm.	cc.	mm.	cc.
1	0?	0?	9.0	0.0495	9?	0.0495
2	0?	0?	7.0	0.0385	7?	0.0385
3	0?	0?	8.0	0.044	8?	0.044
4	1.5?	0.0082	11.5	0.0632	10.0?	0.055
5	3?	0.0165	17.0	0.0935	14?	0.077
6	4?	0.022	25.0	0.1375	21?	0.1155
7	5.5	0.0302	34.0	0.1875	28.5	0.1567
8	6.0	0.033	41.0	0.2255	35.0	0.1925
9†	6.0	0.033	43.0	0.2365	37.0	0.2035
10	6.0	0.033	43.0	0.2365	37.0	0.2035
11	6.0	0.033	41.0	0.2255	35.0	0.1925
12	6.5	0.0357	44.0	0.242	37.5	0.2062
13	6.0	0.033	46.0	0.252	40.0	0.220
14	6.5	0.0357	50.0	0.275	43.5	0.2392
15	6.5	0.0357	55.0	0.3025	48.5	0.2667
16	7.0	0.0385	58.0	0.3190	51.0	0.2805
17	7.0	0.0385	62.5	0.3437	55.5	0.3025
18	7.5	0.0412	67.0	0.3685	59.5	0.3272
19	8.0	0.044	70.0	0.3850	62.0	0.3410
20						
21	8.5	0.0467	72.0	0.3960	63.5	0.3492
22	9.0	0.0495	71.0	0.3905	62.0	0.3410
23	9.5	0.0522	71.5	0.3932	62.0	0.3410
24	9.5	0.0522	72.5	0.3987	63	0.3465
25	9.0	0.0495	76.0	0.4180	67	0.3685
26	8.5	0.0467	82.0	0.4510	73.5	0.4042
27	8.5	0.0467	85.0	0.4675	76.5	0.4207
28	9.0	0.0495	88.5	0.4876	79.5	0.4372
29	9.5	0.0522	91.5	0.5032	82.0	0.4510
30	10.0	0.055	92.5	0.5087	82.5	0.4537
31	10.5	0.0577	92.0	0.5060	81.5	0.4482
32	12.5	0.0687	91.0	0.5005	78.5	0.4317
33	14.0	0.077	91.5	0.5032	77.5	0.4262
34	16.0	0.088	93.5	0.5142	77.5	0.4262
35	19.0	0.1045	96.5	0.5307	77.5	0.4262
36	22.0	0.121	100.0	0.5500	78.0	0.4290
37	25.5	0.1402	104.0	0.572	78.5	0.4317
38†	34.5	0.1897	106.5	0.5857	72.0	0.3960
39	38.5	0.2117	107.0	0.5885	68.5	0.3767

TABLE IV—Continued

N.O. OF PULSE*	ELEVATION AT END OF DIASTOLE	VOLUME AT END OF DIASTOLE	ELEVATION AT CREST OF PULSE	VOLUME AT CREST OF PULSE	AMPLITUDE OF OSCILLATIONS	VOLUME OSCILLATION WITH ANACROTIC LIMB
	mm.	cc.	mm.	cc.	mm.	cc.
40	45.0	0.2475	107.0	0.5885	62.0	0.3410
41	49.0	0.2695	107.0	0.5885	58.0	0.3190
42	52.5	0.2887	108.0	0.5940	55.5	0.3052
43	57.0	0.3135	109.0	0.5995	52.0	0.2860
44	60.0	0.33	110.0	0.605	50.0	0.275
45	62.0	0.3410	112.5	0.6187	49.5	0.2723
46	63.0	0.3465	114.0	0.627	51.0	0.2805
47	64.0	0.3520	115.0	0.6325	51.0	0.2805
48	66.0	0.363	115+	0.6325	49+	0.2695
49	67.0	0.3685	115+	0.6325	48+	0.2640
50	69.0	0.3795	115+	0.6325	46+	0.2530
51	71.0	0.3905	114.5	0.6298	43.5	0.2392
52	71.5	0.3932	115.0	0.6325	43.5	0.2392
53	72.5	0.3987	115.0	0.6325	42.5+	0.2337

* Pulse rate is 174. The rate of fall of pressure averages 1.4 mm. Hg. per pulse; it is therefore somewhat faster in the vicinity of the first signal, and somewhat slower in the vicinity of the second signal.

† First sound.

‡ Sound fainter.

its inception with the very first of the 4th phase sounds, and which manifests itself in the form of a greatly accelerated increase in the volume of blood in the compression chamber, is the failure of the arterial walls to meet during diastole. The potency of this new factor is indicated by the relatively large volume change with which it is ushered in. Thus whereas during the 37 pulses that precede its appearance the basal volume of the artery increases 0.1402 cc., the volume increase with the very first pulse of the new gradient alone amounts to 0.0495 cc. It should be added that the record as reproduced here does not give an accurate conception of the true gradients of the volume changes. The record was made by the method of continuous escapement, in which the pressure on the artery falls at a constantly diminishing rate. Therefore the actual gradients are somewhat steeper in the latter parts of the record relative to those of the earlier parts. It is not necessary to correct the record for this effect because the correction would only serve to exaggerate the significant features of the experiment as shown in the record.

The systolic volume at first is small and increases very slowly, though in the case of figure 18 the initial compression was not quite high enough to bring out this feature clearly. The volume then increases, very rapidly at first (5th to 8th pulses inclusive), and then more and more slowly to the end of the record. These volume changes resemble so closely those drawn into figure 4 as to render further discussion unnecessary; their probable causes can easily be deduced by bearing in mind the discussion of the subject in connection with the development of that figure.

The maximum volume oscillation was registered with the 30th pulse, though it is quite probable that if the even course of the record had not been disturbed at this time by a respiratory wave of blood pressure, it would have been recorded somewhat later. In any event, it is the 2d gradient of the base line, which, we have inferred, is due mainly to the extension of the lower conical closure, that causes the diastolic volume to rise more rapidly than the systolic volume and so determines the appearance of the maximum oscillation at this time. As a matter of fact, however, the reduction of the volume oscillations from this, the 30th, pulse to the last pulse of the 3d phase (37th) is so slight, being only a little over 4 per cent, as to be almost negligible, especially in view of the fact that the diminution in volume with the very next pulse amounts to about 8.3 per cent.

Importance of the lower conical closure as determined by occlusion of the artery

That the 2d gradient of the diastolic volume, as seen in figures 4 and 18, might be determined by the growth of the lower conical closure is supported by some experiments in which the effect upon the compression pulse of occluding the artery some distance below the compression chamber was studied. In these experiments the compression oscillations were recorded by a Frank mirror capsule attached to the author's sphygmomanometer. The method of intermittent escapement was used. At each of the successive decrements a record was made first with the artery open, then occluded and finally open again. The data collected in Table V show that occlusion of the artery at compressions lying below the systolic pressure determine an elevation of the base line. This, it will be recalled, could be due only to an increase in the volume of the artery in the compression chamber. Such an increase in the basal volume might be caused either by a general

rise of blood pressure, which would manifest its effect through the upper conical closure, or by an increase in the peripheral pressure which would manifest itself mainly through an extension upward to the lower conical closure. That the first factor is, however, not of any considerable significance, is indicated by the observation (a) that the elevation of the base line is almost as marked, though much more gradual, in the higher ranges of systolic-diastolic compression as in the lower ranges, and (b) that the elevation of the base line is very slight when the artery is closed while under compressions lying below the diastolic pressure; for occlusion under the former conditions could cause little if any rise of the central pressure, the artery already being practically shut at that time by the compression itself, yet a considerable

TABLE V

Showing the rate of elevation of the base line when the artery is occluded while recording the oscillations in the compression chamber by the method of intermittent escapement (tambour hole closed)

COMPRESSION PRESSURE IN TERMS OF SOUND PHASES	MAXIMUM ELEVATION	NO. OF PULSES ELAPSING
	<i>mm.</i>	
1st Phase	3—	6—
2d Phase	3—	4
Late 2d Phase	3—	2
3d Phase	3+	2
Last 3d Phase	3++	1
4th Phase	1	1

elevation of the base line occurs. On the other hand, occlusion under the latter conditions probably does increase the central pressure, but it causes only a slight elevation of the base line. Still it is probable that the effect of occlusion of the artery upon the diastolic volume is not manifested wholly through the lower conical closure, for if it were, it would seem that the effect should diminish somewhat with decompressions within the systolic-diastolic range, and it does not. Furthermore, the elevation of the base line in this experiment amounts to 20 per cent of the amplitude of the maximum oscillation. In the experiment recorded in Table IV the 2d basal gradient lifts the base line (0.1402-0.0687) 0.0715 cc., or 16 per cent of the amplitude of the maximum oscillation. These figures are near enough alike, considering the fact that they are taken from different experiments, to signify that the

additional factor in the occlusion experiments determining an increase of volume is not very considerable.⁸

The experiment also shows, by the much more rapid distension of the artery when it is occluded in the later systolic-diastolic stages, that under usual conditions the influence of the lower cone must be far more significant than during the earlier stages. The peripheral resistance, of course, will be of considerable significance in this connection.

Form of the artery in relation to the maximal oscillation

As shown by volume records. Special attention is directed to the fact that the maximal oscillation (30th pulse of fig. 18) is not recorded while the artery is in the "half-flattened" position during diastole. As a matter of fact the diastolic volume at this time has increased only (0.0577-0.033) 0.0247 cc., above the diastolic volume under systolic compression. This is less than one-sixth of the volume increase during the whole of the systolic-diastolic range, and considerably less than the volume increase (over 0.04 cc.) associated with each pulse at a time when the artery is completely collapsed during the entire pulse cycle (1st to 5th pulses). Indeed, the elevation of the diastolic volume, as we have already indicated, is attributable rather to the extension of the upper conical closure than to any opening of the middle region of the compressed artery. The records thus obtained from animals, therefore, completely confirm the results obtained under the ideal conditions supplied by a properly constructed and properly operated circulation schema. In view of the fact that it is possible, on the basis of the configuration of the pulse they used, to account satisfactorily for the finding of MacWilliam and Melvin to the effect that the artery in their circulation schema oscillated from the "half-flattened" position rather than from the flattened position while maximum oscillations were recording, there is no longer any good reason for doubting the earlier observations on this subject. Indeed, there is now every reason for holding, as we have held in the past, that under the conditions that obtain in practical sphygmomanometry, barring possibly a loss in the transmission of the pressure to the artery, the arterial walls continue to meet during diastole until the compressing pressure falls below the diastolic pressure.

⁸ The additional factor will be considered in a subsequent paper.

As indicated by the time elapsing between the beginning of the compression and peripheral pulses. If the compression pulse and the pulse in the artery just below the compression chamber be recorded simultaneously by Frank capsules it is found that with diminishing compression pressure the interval between the beginning of the compression pulse and of the peripheral pulse diminishes more or less steadily and relatively rapidly until the 3d phase sounds cease (see Tables II and III). Beginning with the 4th phase sounds the interval remains fairly constant or continues to decrease, though at a very much slower rate. Between the last of the 3d phase and the first of the 4th phase pulses this diminution in the time interval is the greatest that occurs in this particular region of the record. It seems fair to assume in explanation of this phenomenon that as long as the time interval is diminishing at a relatively rapid rate the artery is opening from the collapsed state earlier and earlier in the pulse cycle; that the marked diminution in the interval usually observed with the beginning of the 4th phase sounds marks the moment when the walls first fail to meet during diastole; and finally that the slower subsequent diminution in the interval in part at least is an expression of the increase in the coefficient of elasticity of the artery as diminishing compression permits the tension peripherally to increase.

Observations bearing on the time the arterial walls first begin to separate during decompression

A series of observations was also made in an effort to ascertain the moment, in relation to the compressing pressure and arterial pressure, the arterial walls first begin to open out under a compression that is falling from above the systolic arterial pressure. It was found by trial that the following was the most delicate method for making these observations. The bare femoral artery was closed by compressing it in the arteriograph. A long, straight, narrow glass tube was then inserted into the artery some distance below the arteriograph and by momentarily lowering the pressure on the artery blood was permitted to flow under the obstruction until it appeared in the tube. Then, beginning well above the systolic level, the compressing pressure was permitted to fall *very slowly* and the compressing pressure was read by an assistant as the observer, who could not see the manometer, announced (a) when the blood began to move in the tube, (b) when this progression first became distinctly pulsatile, (c) when it became decidedly pulsatile and (d) when the first sound was heard. After each observa-

tion the tube was flushed out with carbonate solution in order to prevent clotting. Somewhat greater accuracy would have been attained if the compressing pressure had been recorded and the observer had signaled the readings on the same record. The fact, however, that the compressing pressure was falling very slowly really made that method unnecessary.

TABLE VI

Showing the character of the blood flow through the compressed artery during the early stages of decompression

1 DOG NO.	2 BLOOD FLOWS	3 PULSE APPEARS	4 SOUND APPEARS*	5 DIFFERENCE 2-4
	mm. Hg.	mm. Hg.	mm. Hg.	mm. Hg.
15	142	134	128	14
	142	134	132	10
	141	136	129	12
	142	136	132	10
	142	137	132	10
	140	134	130	10
	146	138	136	10
Average....	11
16	114	110	106	8
	114	111	108	6
Average....	7
17	148	146	140	8
	148	146	132+	16
	148	145	136	12
	148	144	134	14
Average....	12.5

* Also brusque pulse.

It is seen in the Table (VI) giving the results of these observations that the brusque pulsatile progression of the blood and the first sound invariably make their appearance simultaneously. It should be added that the change in the character of the pulsatile progression at this time is sudden and unmistakable. They appear on the average from 7 to 12 mm. of mercury below the compression under which the column of blood first begins to move. The earliest obvious pulse appears under intermediate compressions.⁹ The figures giving the appearance

⁹ This pulse must be exceedingly feeble, for only rarely can it be felt; and our method of recording the pulse, which was probably not quite as delicate as the finger, has never brought it out.

of the pulse and of sound vary considerably and the latter more than the former, relative to those indicating the pressure under which the blood begins to flow. This, we believe, is not due to any differences in the sharpness of the criteria, but rather to the fact that pulsation and the sounds are more apt to show the effects of the pressure changes associated with each individual pulse than is the beginning of flow, which probably is determined by small accretions from many pulses.

Be this as it may, the essential facts brought out by these observations are that the blood begins to pass through the artery, though exceedingly slowly, under a compression that exceeds the level at which the first sounds are heard by from 7 to 12 mm. of mercury; and that in the latter part of this stage the flow may become slightly pulsatile. It might be mentioned in passing that the compression pulse of the bare femoral artery of the dog recorded by a Frank capsule on a rapidly moving surface shows the effect of this passage of blood through the artery, and that it becomes obvious, as does the passage of blood, at compressing pressures about 10 mm. of mercury above the level at which the first sound is heard.

The fact that blood and even a "rudimentary" pulse may begin to pass beneath the compression before the so-called "fully developed" pulse (8,3) has been known for some time. The present experiments prove, however, that the artery first opens by orifices so narrow that the pulse is entirely lost in them. These must be orifices formed by folds in the wall of the collapsed artery, for if the first blood that succeeds in getting through were forced through by the opening of the artery by the pulse, it would show a pulsatile progression from the very first. The later faint pulse may be taken to indicate that these orifices are now large enough to let through some of the pulse. But the abrupt appearance of the brusque pulse indicates the entrance of a wholly new phenomenon. This new phenomenon could be but one thing, namely the separation of the walls of the artery throughout their entire periphery, and not merely where they are creased.

Effect of the size of the compression chamber in animal observations

It is not difficult to show that in observations on animals the compressibility of the compression space and the phase of the pulse cycle in which the compressibility of the space is reduced have consequences quite similar to those seen in the experiments with the circulation schema. As, however, the results obtained in the animal experiments must be considered in detail in connection with the mechanism of sound production in arteries, it will not be necessary to discuss them now.

SUMMARY

Experiments are described which were designed primarily in an effort to harmonize the conflicting views that are held with regard to the significance of the critical pressure oscillations yielded to a space through which an artery is being compressed. At the same time it was hoped that additional information in regard to the mechanism of the compression oscillations might be brought to light.

Experiments have been performed both on rubber tube and artery in a circulation schema operated by a method of procedure described by Brooks and Luckhardt, and on arteries in situ. The results have been as follows:

1. In the case of one and the same tube or artery the general configuration of the record of the compression pulses depends upon (a) the compressibility of the compression space and, if that is sufficiently small; upon (b) the phase of the pulse cycle in which the compression space is closed. The variations in configuration may be so marked under the different conditions as to show maximum oscillations at systolic, diastolic or mean compression pressures. Some of the discrepancies in the views held with regard to the significance of critical oscillations undoubtedly are attributable to the differences in the experimental conditions enumerated above.
2. The configuration of the oscillation record is influenced also by the extensibility of the artery, by the significance of the upper and lower conical closures of the artery relative to that of the completely occluded part between (length of artery), and by the relation of the volume of the undistended bore to that of the distended bore of the artery in the compression chamber.
3. When the compression space is sufficiently large the compression oscillations are proportional to the volume changes of the artery produced by the pulse and to the compressing pressure.
4. The volume change with each pulse is then determined by the difference between the volume of blood in the artery under the compression chamber during diastole and during systole.
5. During decompression the diastolic volume increases constantly though along three successive gradients, each of which is in the main determined by a different process, namely (in the order of their appearance): (a) the descent of the upper conical closure of the artery, (b) the ascent of the lower conical closure, and (c) the filling of the intermediate segment of the artery to its undistended bore (at diastolic

compression) and the subsequent stretching of the arterial walls at compression below diastolic pressure.

6. The systolic volume also increases constantly and along three gradients determined by the following processes respectively: (a) the descent of the upper conical closure, (b) the filling of the central segment to its undistended bore (at systolic compression) and (c) the subsequent stretching of the arterial wall.

7. The diastolic and systolic volume gradients are so related to each other that the compression oscillations determined by their separation in different stages of decompression have the relative amplitudes usually seen in records of the blood pressure made by the oscillatory method; though it is obvious that as a result of differences in the relative significance of the factors determining the systolic and diastolic gradients variations from the typical record must frequently occur. Thus the slight diminution in the amplitude of the oscillations frequently observed before the sudden diminution begins is attributable mainly to an increase in the influence of the lower conical closure of the artery.

8. However under all circumstances, natural as well as artificial, a sudden increase and a sudden decrease in the amplitude of the oscillations, if present, indicate accurately the systolic and diastolic pressures respectively.

9. It is shown that with a pulse of the configuration of the arterial pulse the maximal oscillation must be and is recorded at a time when the artery is still collapsed in the diastolic phase of the pulse cycle by the pressure from without. The maximal oscillation can be obtained at a time when, during decompression the artery has attained the "half-flattened" state (MacWilliam and Melvin) only if the pulse has an atypical form, such as probably could be developed under artificial conditions only.

10. During decompression a slight flow of blood, which soon becomes faintly pulsatile, begins about 10 mm. of mercury above the compressing pressure at which a brusque pulse, undoubtedly marking the first opening out of the artery from the "collapsed" state, appears.

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THE EFFECT OF NICOTINE UPON THE REFLEX ACTION OF SOME CUTANEOUS SENSE ORGANS IN THE FROG

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The basis of this investigation was to determine the effect of nicotine upon certain skin reflexes in the frog: to determine the duration of this effect; its after effect; if immunity could be established; and how the action of nicotine compares with that of alcohol. The experiments were conducted upon frogs of the species *Rana Pipiens*, secured from a nearby lake. They weighed from 15 to 120 grams, and during the period of experimentation were kept in a dark room in cool moist moss, without food.

The sensory ending of the cutaneous nerves may be considered peripheral end organs. By carefully testing certain spots, some were found which responded with a fairly constant reflex time, to a definite stimulus. The same spot varies somewhat at different times of the day and also at different room temperatures; on cool days the response was quicker.

The spots selected for study were those which proved after careful testing to be most reliable and constant in their reaction time. These spots are illustrated in text, figure 1, and were the same ones employed in a former experiment (1).

The constant stimulus determined upon, was one that would not injure or fatigue the peripheral nerve endings during the period and method of experimentation. Pure neutral filter paper, three millimeters square, moistened with 8 per cent pure acetic acid was found the most practical chemical stimulus. The paper was moistened in the acid, then placed carefully by means of a long forceps upon the spot to be tested. That the errors due to sight and pressure stimuli were avoided, was proved by control experiments in which the eyes were covered by a special device.

By reflex time is meant the interval between the moment the paper touches the skin and the moment the frog made an attempt to remove it. It was found that if the attempt was not made within one minute, it usually never would be. As soon as an attempt was made or if not made at the end of one minute, the acid paper was washed off with fresh water thus preventing fatigue and injury to the peripheral nerve ending. This was proved by testing the corresponding spot on the

opposite side and also by control experiments on frogs which had not been given nicotine.

The nicotine used in these experiments was dissolved in distilled water and injected into the dorsal lymph sac. The doses employed varied from $\frac{1}{2}$ minim of 0.05 per cent to 27 minims of 0.1 per cent nicotine per 10-gram frog. Control experiments were made by injecting Ringer's solution in amounts equal to the maximum amount of fluid introduced with the drug. These control experiments served to check the mechanical effect of injection and the effect of dilution on the circulating fluid.

In carrying out an experiment, the reflex time of the series was found upon the frog before nicotine was given, then the drug was injected, and 10 minutes were allowed to overcome the mechanical effects before testing the spots again. This testing was repeated every ten minutes for about two hours or until the reflex response returned, allowing an interval of 10 minutes rest between each test. If the reflex response did not return at

the end of two hours, the tests were repeated the following day. The acid paper was applied with one hand and the stop watch was used with the other to record the reaction time. Philips and Pembrey (2) give 10 minims (0.59 cc.) of 1 part of nicotine in 20 parts saline solution as a toxic dose and Cushny 1 cc. of 0.2 per cent solution. The reflex time and effects secured after injection of nicotine, were compared with the reaction time and effect obtained before injection, also with the reaction time after injection of Ringer's solution.

It was found that each spot had its own reflex time, its own degree

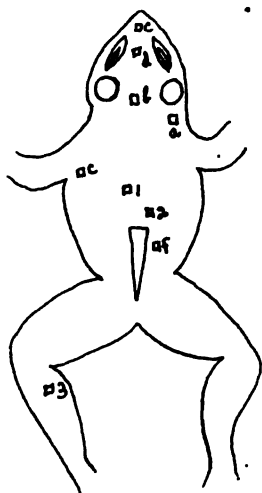


Fig. 1. Diagram showing location of sensory spots on the frog. Those on the head are innervated by branches from the cranial nerves, those on the trunk and legs by spinal nerves.

of irritability and that some were more resistant to nicotine than others. Spot C (fig. 1 and Table I) is normally irritable, usually responding to the acid stimulus in one second, and after doses large enough to cause a loss of reflex, was always the first to recover; often being the only spot to give a reaction response. Spots f and c also respond quickly to the acid stimulus and recover quickly from the seemingly paralyzing effect of the nicotine. Spots 1 and e seem to be the least irritable

TABLE I
Comparison of irritability of spots

SPOTS	I REACTION TIME BEFORE NICOTINE	II REACTION TIME AFTER NICOTINE	III LENGTH OF PARALYZING EFFECT		
			0.5 m. (a)	5 m. (b)	14 m. (c)
1.....	2 seconds	4 seconds	40 minutes	100 minutes	180 minutes
2.....	12 seconds	20 seconds	40 minutes	100 minutes	180 minutes
3.....	2 seconds	4 seconds	20 minutes	80 minutes	120 minutes
a.....	7 seconds	8 seconds	20 minutes	100 minutes	360 minutes
b.....	8 seconds	38 seconds	40 minutes	150 minutes	360 minutes
c.....	1 second	1 second	10 minutes	50 minutes	120 minutes
d.....	5 seconds	5 seconds	20 minutes	50 minutes	120 minutes
e.....	15 seconds	30 seconds	40 minutes	150 minutes	360 minutes
f.....	4 seconds	10 seconds	20 minutes	75 minutes	180 minutes

For location of spots, see text, figure 1.

M. Minims, 1 drop, 0.16 cc.

Stimulus-acid paper, see text.

Nicotine of 0.05 per cent.

Column I shows reaction time to an acid stimulus for a normal frog.

Column II shows the immediate depressing effect of nicotine after paralyzing effect has passed off. e. g. Spot I in a normal frog responded to the acid stimulus in 2 seconds after the paralyzing effect, that lasts for 40 minutes, has passed off, its reaction time to the stimulus increased to 4 seconds.

Column III shows the length of time that the paralyzing effect of a weak, medium, and strong dose lasted for the different spots.

and show the least resistance to the action of the drug. It was found that spots c, 3, and f were the most irritable normally and showed the most resistance to the action of the drug. Table I also shows that nicotine has both a paralyzing and a depressing effect. First it paralyzes the ganglia cells (according to Langley), then as soon as the paralyzing effect passes off, there is a depression of the spot, making it slower in its response to the acid stimulus shown in Column II and I. Column III shows the duration of paralysis in the different spots,

due to the doses indicated. For instance spot c lost its reaction time for 120 minutes, while b lost it for 360 minutes.

It was interesting to note that as weak a dose (Table I, III a) of nicotine as $\frac{1}{2}$ minim of 0.05 per cent per 10-gram frog produced a change in the reflex time of the cutaneous sensory spots, but no apparent effect on other reflex actions, such as turn over, compensatory, and swimming, or upon the general behavior of the frog. It had a more depressing effect than a larger dose of Ringer's solution. With this dose, spot c was the only one which did not lose its irritability or fail to respond to the acid stimulus at any time. Small doses from $\frac{1}{2}$ to 5 minims per 10-gram frogs were followed for about 15 minutes by slightly forced or labored breathing, and also by a slight constriction of the pupils. Weak fibrillary twitchings were also noticed immediately after injection. These lasted only for a short time, during which the position was usually normal.

With medium doses (Table I, III, b) the skin reflexes were all lost and for a longer time, the breathing became more forced, the constriction of the pupils more apparent, and the fibrillar twitchings more pronounced. The frog assumed a flattened position, and even after the deepened breathing became normal again, seemed sluggish.

With large doses (Table I, III a) i.e., of 10 minims of 0.05 per cent per 10-gram frog and over, the higher reflexes as well as skin reflexes were lost for 1 hour or longer. It was noticed that when the spots failed to respond after a dose of nicotine, they did so immediately. The paralyzing effect increased with increase of the dose. The frog returned to a seemingly normal state within two days, but the skin reflexes usually displayed an increased irritability for some time. There was a period of inhibition of respiratory movements at first, the length increasing with an increase of dose. With the largest doses, this inhibition lasted for two hours or longer, and was followed by forced irregular breathing.

The large doses also caused an immediate stiffening or tetanus of the front legs, which lasted about 15 minutes. This stiffening spread somewhat to the trunk and hind limbs, then was followed by a relaxation and loss of muscle tone, and continued until the higher reflexes again made their appearance. From these observations we conclude.

1. That nicotine causes a loss of the skin reflexes, a seemingly paralyzing effect, that is followed by one of depression.
2. That nicotine in small doses causes forced breathing; in large doses an inhibition of respiratory movements, followed by forced irregular breathing.

3. That it causes fibrillar twitchings and constriction of the pupils.
4. That large doses cause tetanus contraction of the front legs, and sometimes a slight stiffening of the whole body, followed by relaxation and loss of muscle tonus.

II. TOLERANCE EXPERIMENTS

In connection with the above observations, a series of experiments were carried on to determine if tolerance to nicotine could be established. A number of frogs were given first a minimum dose, $\frac{1}{2}$ minims of 0.05 per cent per 10-gram frog, then at intervals of two days were again injected with doses of one minim until the last dose given proved toxic to a normal frog. To prove that laboratory conditions, and fatigue due to acid tests, were not factors to be considered, control frogs were placed under the same laboratory conditions, and subjected to the acid tests as frequently, and on the same spots, as were those employed in the tolerance experiments.

Table II shows the typical effects of equal doses of nicotine upon a normal frog and upon one in which tolerance is being established, by giving it gradually increasing doses. In comparing the effects of nicotine on these frogs it was seen that with small doses, there was considerable difference in the effect upon the two frogs. The normal one lost all the skin reflexes for a time and exhibited fibrillar contractions. The only apparent effect upon the tolerant frog was the forced breathing and increased irritability. Its skin reflexes all responded to the acid stimulus at the end of 10 minutes. With medium doses 10 minims of 0.05 per cent to 7 minims of 0.1 per cent per 10-gram frog, the most noticeable difference was that, although both frogs passed through the different stages produced by nicotine, they did not last so long in the tolerant frog, and its skin reflexes responded much more quickly to the acid stimulus. It was interesting to see that sometimes the skin reflexes responded even before the higher reflexes returned, this being contrary to the usual events.

Other interesting observations taken on the tolerant frog were, that after recovery from the effect of the drug the animal became very irritable and the spots responded abnormally, all of them within one second. Often the slightest touch on the skin called forth a violent reflex movement. The tolerant frog also began to show a yellow discoloration of the skin on the under side of the lower part of the body and hind legs. Although this discoloration remained permanent, it was most noticeable shortly after injection. It was also found, that there

TABLE II
Comparison of normal and tolerance frogs

*FROG	DOSE PER 10 GRAMS	% NICO- TINE	SKIN REFLEXES LOST	HIGHER REFLEXES LOST	IRRITABILITY	CONSTRUCTION OF PUPILS	POSITION	BEHAVIOR	RESPIRATION
Normal....	5 m	0.05	15 min.	No loss	None	Very slight	Normal	Normal	Deeper
Tolerance...	5 m	0.05	No loss	No loss	Very slight	Very slight	Normal	Normal	Deeper
Normal....	3 m	0.05	32 min.	No loss	None	Slight	Not normal	Slight twitchings	Forced
Tolerance...	3 m	0.05	No loss	No loss	Slight	Slight	Not normal	Normal	Forced
Normal....	6 m	0.05	More than 2 hrs.	No loss	None	Constricted	Flattened	Twitchings	Forced
Tolerance...	6 m	0.05	No loss	No loss	Quiet	Constricted	Slightly flattened	Normal	Forced
Normal....	10 m	0.05	64 hours	60 min.	Sluggish	Very constricted	Very flat	Twitching	Inhibition, fol-
Tolerance...	10 m	0.05	83 min.	60 min.	Sluggish	Very constricted	Very flat	No twitching	lowed by irreg- ular
Normal....	14 m	0.05	More than 12 hrs.	More than 4 hrs.	Sluggish	Very constricted	Stiffening of front legs	No twitching	Inhibition at first
Tolerance...	14 m	0.05	125 min.	95 min.	Loss of mus. tonus	Very constricted	Stiffening of front legs	No twitching	then irregular
Normal....	18 m	0.05	More than 12 hrs.	More than 6 hrs.	Not very	Very constricted	Very flat	Twitchings follow injections	Apparent inhibi- tion for some time
Tolerance...	18 m	0.05	More than 12 hrs.	More than 6 hrs.	Very irritable	Very constricted	Very flat	Twitchings follow injections	Apparent inhibi- tion for some time
Normal....	7 m	0.1	About 12 hrs.	More than 6 hrs.	Not very	Very constricted	Very flat	Twitchings follow injections	Inhibited followed by irregularity
Tolerance...	7 m	0.1	Never returned	Never returned	Lost	Very constricted	Very flat	Twitchings follow injections	Never recovered
Normal....	9 m	0.1	Never returned	Never returned	Lost	Very constricted	Very flat	Twitchings follow injections	Never recovered
Tolerance..	9 m	0.1	Never returned	Never returned	Lost	Very constricted	Very flat	Twitchings follow injections	Never recovered

was a decided loss of weight of the frogs which were given frequent doses of nicotine, while the control frogs kept under the same conditions showed but slight loss, due perhaps to lack of food. As the toxic dose was reached, the tolerant frog seemed to become weaker and its power of resistance lessened.

Some of my results corroborate those published by Langley (3), who found that in the skate, 1 per cent nicotine has an extraordinarily strong and local effect upon the bulb and cells in the sympathetic ganglia, also that the application of nicotine to the spinal bulb causes for a time, cessation of the respiratory movements in consequence of the tetanic contraction of the muscles; the muscles then relax and feeble respiratory movements occur. He explains the fibrillary twitchings by the facts that nicotine is a stimulant to the motor nerve cells of the bulb and spinal cord. After this stimulation, however, there is a paralyzing effect. I also found as did Cushny (4) and Sollman (5), that nicotine caused a constriction of the pupil.

In comparing the results exhibited by nicotine with those obtained in a previous investigation with alcohol (6), I found that the differences are quite marked. Both cause a loss of the skin reflexes and in sufficient quantities, loss of the higher reflexes, but nicotine is much more powerful. A dose of 0.008 cc. of 0.05 per cent nicotine per 10-gram frog affects the animal more intensely than a dose of 0.005 cc. of 15 per cent to 30 per cent alcohol per 10-gram frog. Nicotine produces a slight stimulation at first, alcohol never stimulates. Alcohol did not affect the respiration or cause constriction of the pupils. But in large doses, it too caused tetanic contraction of the whole body and death. Nicotine causes a peculiarly characteristic tetanus of the front legs followed by a relaxation and loss of muscle tonus.

This investigation was undertaken at the suggestion of Dr. Ida H. Hyde, to whom I am greatly indebted for constant supervision and help.

SUMMARY

1. Certain sensory spots in the frog's skin differ not only in irritability and reflex action, but also in susceptibility to the influence of nicotine.
2. The skin reflexes are affected by much smaller quantities of nicotine than are the higher reflexes, turn-over, compensatory, and swimming.
3. Small doses of nicotine cause a depression or loss of the reflexes, fibrillar contractions, forced respiration and a slight constriction of

the pupils. One dose did not usually show an increased irritability as an after effect. These changes appear immediately after injection, and last from one-half to three or four hours, depending upon the dose.

Doses of $\frac{1}{4}$ minim of 0.05 per cent per 10-gram frog have a greater effect than larger doses of Ringer's solution. Large doses of nicotine cause an entire loss of the skin reflexes, and muscle tone, producing a flattened position; an inhibited then irregular respiration; constriction of the pupils; loss of turn-over, compensatory and equilibrium reflexes; a tetanus or stiffening of the front legs, followed by a relaxation and loss of muscle tonus.

4. Continued gradually increasing doses of nicotine cause tolerance to the drug, such that it loses some of its effect. Continued use of nicotine causes increased irritability of the skin reflexes, making them respond abnormally. It also causes a loss of weight, and a discoloration of the skin. The effect upon respiration and constriction of pupils is the same as in a normal frog, except that the effect does not last so long.

5. The toxic dose obtained from the results of a large number of experiments, showed that it varied from 6 to 27 minims of 1 per cent nicotine per 10-gram frogs. Some frogs naturally proved more resistant than others. For those frogs which had been given continued gradually increasing doses, the toxic injection varied from 6 to 9 minims of 1 per cent per 10-gram frog. Nicotine acted as an accumulative drug lessening the bodily resistance until the toxic dose is found to be less for the tolerant frog than for the normal one.

6. Nicotine is much more powerful in its effects on cutaneous reflex reactions, ciliary muscles, respiratory activity and bulbar centers than is alcohol.

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THE COAGULATION OF BLOOD IN THE PLEURAL CAVITY

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The fact that blood in the pleural cavity remains fluid or partially fluid and that such blood fails to coagulate after withdrawal has been shown experimentally by Penzolt (1), Pagenstecker (2), and more recently by Zahn and Walker (3). It has also been observed clinically that blood in the pleural cavity generally remains fluid or partially so and does not clot when withdrawn.

The experiments of Zahn and Walker show that small amounts of blood (5 to 8 cc.) introduced slowly into the pleural cavity either from the internal mammary artery or by means of a syringe, remain fluid. To obtain this result they lay stress on the fact that small amounts must be injected and that deep artificial respiration must be maintained. Such blood when withdrawn from the pleural cavity some 10 to 20 minutes after injection can not be coagulated by addition of thrombin, calcium or thromboplastic extracts. By the heat test fibrinogen was not detected and it was only by the addition of a fibrinogen solution that the blood could be coagulated. Since these authors were unable to find any fibrin in the pleural cavity either microscopically or macroscopically they concluded that the fibrinogen was in some way altered or destroyed by contact with the pleural endothelium. They were, however, unable to show that extracts of this endothelium had any retarding action on the coagulation of whole blood in the test tube.

We have repeated their experiments on dogs many times using their technique. In our most successful experiments, the blood was found to be almost entirely fluid but in all cases we found a small clot generally situated about the hylus of the lung. When injections of blood were given more rapidly and in larger amounts there resulted a relatively larger clot with a smaller amount of fluid blood.

We found, as did Zahn and Walker, that the fluid portion of the blood gave no precipitate on heating to 60°C. and that it would not clot on

addition of thrombin,¹ calcium² or thromboplastic solutions.³ The addition of fibrinogen⁴ caused a rather poor clot to form after some hours.

We also determined the amount of anti-thrombin⁵ in the fluid portion of the blood and found no appreciable difference between it and a sample of oxalated plasma taken from the same blood used for injection.

Although no increase in antithrombin was found, serum and Ringer's solutions were introduced into the pleural cavity and allowed to remain 10-30 minutes with the hope that they might acquire some anti-coagulating power from the pleural surfaces. This hope was not realized. When tested on whole blood both solutions hastened coagulation in all instances.

The fact that Ringer's solution accelerated coagulation after being in the pleural cavity was probably due to admixture with small amount of serum or pleural exudate containing thromboplastic material. In almost all instances we recovered more fluid from the pleural cavity than was injected. Some of this excess was probably serum or pleural exudate containing thromboplastic material, which would accelerate coagulation when added to whole blood.

As is mentioned by Lord (4), the question has been raised as to whether the failure of these bloods to coagulate is due to a destruction or alteration of fibrinogen by the pleura or to a previous coagulation and defibrination in the pleural cavity.

In order to determine whether fibrinogen alone is altered or destroyed by contact with pleural surfaces the following experiments were made.

FIBRINOGEN INJECTION

Injections of 10 to 30 cc. of fibrinogen (prepared according to a modification of Hammarsten in method) were made into the pleural cavities of dogs, seven such experiments being done. The usual precautions of slow injection and deep artificial respiration were observed and the solutions were allowed to remain in the chest 10 to 30 minutes.

In one instance a small membranous clot was found on opening the chest but in the other six the fluid was clear or slightly blood tinged.

¹ Thrombin prepared according to Howell's method. This Journal, 1913, xxxii, 264.

² 0.5 per cent solution of delequescent CaCl_2 .

³ Kephalin or fresh spleen extract. See Howell, This Journal, 1912, xxxi, 1.

⁴ Prepared by a modification of Hamarsten method.

⁵ Howell's method, Arch. Int. Med., 1914, xiii, 76.

In all cases the fibrinogen after withdrawal was clotted much more rapidly by thrombin than control specimens of fibrinogen which had not been injected.

It has already been pointed out, in speaking of the injection of Ringer's solution, that serum or thromboplastic material is probably responsible for this accelerated coagulation. That thrombin was added to the fibrinogen solutions while in the pleural cavity seems likely since if the fibrinogen were allowed to stand some hours after withdrawal a faint veil like clot sometimes formed indicating that a small amount of thrombin was present.

From these experiments it is evident that fibrinogen itself is in no way altered so far as its power to clot with thrombin is concerned by contact with pleural surfaces. In further experiments an attempt was made to determine what occurs when fibrinogen and thrombin are injected together into the pleural cavity.

INJECTION OF THROMBIN AND FIBRINOGEN

Experiment i. 20 cc. of a fibrinogen solution was injected into the pleural cavity and allowed to remain for about 10 minutes. At the end of this time 5 cc. of a thrombin solution was injected and 20 minutes later the chest was opened a large firm clot being found. The proportion of thrombin to fibrinogen in this case was very large, so much so, that the mixture clotted solidly in the test tube in less than 2 minutes.

Experiment ii. Thrombin and fibrinogen were mixed outside the body and 8 cc. of the mixture was immediately injected slowly into the pleural cavity. The proportion of thrombin was much smaller than in Experiment i, a specimen clotting in 10 minutes in the test tube.

After 30 minutes the chest was opened and 14 cc. of fluid was withdrawn. A long thin clot was found back of the root of the lung but the proportion of clot to fluid was small. The fluid portion did not clot on standing. It was not clotted by addition of thrombin and showed no precipitate on heating to 60°C., indicating an absence of fibrinogen. Adding thromboplastin and calcium failed to cause coagulation but the addition of fibrinogen caused a poor clot to form after some hours.

Experiment iii was identical with Experiment ii except that the proportion of thrombin to fibrinogen was even smaller, a specimen clotting in the test tube in 14 minutes. Immediately after mixing $4\frac{1}{2}$ cc. of this thrombin and fibrinogen were introduced very slowly into the pleural cavity and at the end of 30 minutes the chest was opened and $4\frac{1}{2}$ cc. of

fluid withdrawn. No trace of any clot could be seen but no microscopic examination for fibrin was made.

This fluid remained unclotted for several days and could not be coagulated by thrombin, calcium or thromboplastin. Fibrinogen was absent by the heat test and addition of fibrinogen caused the usual slow coagulation. Addition of fibrinogen and thromboplastin caused a clot in 15 minutes.

These experiments with artificial solutions quite parallel those with the whole blood.

SUMMARY AND CONCLUSIONS

Small amounts of blood introduced slowly into the pleural cavity when deep artificial respiration is maintained will remain in large part fluid. Small clots are always found in the pleural cavity, the size depending on the amount of blood injected and the rapidity of injection.

The fluid portion of the blood can only be clotted by addition of fibrinogen. Thrombin, calcium and thromboplastin are incapable of causing coagulation. The blood shows absence of fibrinogen which perhaps may be removed in some way other than by coagulation.

Pure fibrinogen solution which has been in the pleural cavity under the same conditions, not only is not altered, but clots more readily than the control on adding suitable amounts of thrombin.

Small amounts of thrombin and fibrinogen when mixed in suitable proportions and injected slowly into the pleural cavity remain fluid and show an absence of fibrinogen.

Since it has been shown that fibrinogen alone loses none of its properties after remaining in the pleural cavity, and that the presence of thrombin under the same conditions causes a disappearance of fibrinogen, we can only conclude that coagulation has taken place.

The experiments with artificial solutions exactly parallel those with whole blood, the conclusion being that blood which has been in the pleural cavity remains fluid not because of any alteration of the elements, but because of previous coagulation and defibrination.

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GASTRO-INTESTINAL STUDIES

XII. DIRECT EVIDENCE OF DUODENAL REGURGITATION AND ITS INFLUENCE UPON THE CHEMISTRY AND FUNCTION OF THE NORMAL HUMAN STOMACH¹

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INTRODUCTION

In the course of the extensive gastric investigations undertaken in this laboratory it has long been noted that following the introduction of certain substances into the stomach, the color of the samples removed varied from that of the material introduced to a golden yellow or a dark olive or blue green. The color in the gastric contents is said by Sartory (1) to be sometimes due to the *cryptococcus salmonius*, and in certain cases of hyperacidity the blue-green mould *penicillium crustaceum* has been found. But all our samples showing color, on standing exposed to light for one hour became deep green and the oxidation tests for bile pigments were positive. Furthermore, in repeated experiments on the same individual with the introduction of such substances as water, sodium chloride, alkaline or acid solutions, color changes were almost invariably present; while similar experiments with certain other substances as an Ewald test meal, cereals, etc., showed no coloring of the samples. If the color were due to the *cryptococcus salmonius* or the *penicillium crustaceum* its presence should be independent of the material introduced. We, therefore, attributed the color changes in these cases to the presence of bile.

By this evidence of regurgitation of duodenal contents we were led to further investigate the theory propounded by Boldyreff (2, 5) "The self-regulation of the acidity of the contents of the stomach." This theory states that the initial high acidity of the gastric juice, namely, 0.32 per cent to 0.48 per cent HCl as has been proven by recent inves-

¹ Reported before American Physiological Society, by title, December, 1914, and read before American Philosophical Society, April, 1915.

tigators (6, 15), is lowered to the optimum acidity of 0.15 to 0.2 per cent HCl by "an influx of intestinal juices into the stomach with the aim of neutralizing the superfluous acid in it. A portion of the strong acid fluid passing from the stomach into the intestines provokes an abundant secretion chiefly of the pancreatic juice, and, if there is not sufficient pancreatic juice, there are also bile and intestinal juice secreted. The acid fluid, moreover, irritates the intestines, thus provoking antiperistalsis which drives the alkaline secretions of the intestines into the stomach until a sufficient amount accumulates which is capable of lowering the acidity of its contents to the usual level of 0.15 per cent HCl." Migai (16) and Cathcart (17) confirmed the theory and recently Milosorov (18), by work on dogs provided with successive intestinal fistulas, observed that the farther from the pylorus the fistula through which the intestinal contents are removed, the lower the acidity of the gastric contents. Carlson (18a) in speaking of Boldyreff's theory says "I am satisfied from my observations on Mr V., that Boldyreff's view is essentially correct." No data were reported. Hicks and Visser (18b) from observations made under Professor Carlson's direction recently report that "In man, an average of 32.6 cc., of gastric juice (acidity 0.411 per cent) accumulated in twenty minutes chewing of food, causing regurgitation in 40 per cent of ten cases, whereas 100 cc. of 0.4 per cent HCl retained in the stomach for twenty minutes caused no regurgitation in 100 per cent of ten other cases." It is worthy of note that bile was taken as the indicator of regurgitation in the above experiments. Hicks and Visser state that "duodenal regurgitation is not the factor of greatest importance in the reduction of the high acidity of the stomach contents."

If duodenal regurgitation does occur we should be able to recognize some of the constituents of the duodenal secretions in the material removed from the stomach. We have already noted the presence of bile in the specimens of gastric contents, but its occurrence being inconstant renders it rather unserviceable as an indicator of the degree of duodenal regurgitation. Then too, pancreatic amylase, amyllopsin, could not readily be distinguished from salivary amylase, ptyalin, and pancreatic lipase, steapsin, could not be taken, for a gastric lipase has been said to be present (19). Trypsin because of its characteristic property of digesting protein in alkaline media and the readiness with which it may be determined quantitatively should prove the ideal indicator. We, therefore, undertook the quantitative estimation of trypsin in the samples obtained by fractional examination of the gastric

contents in an effort to determine if possible the relation of duodenal regurgitation to the chemistry and function of the stomach.

Ehrenreich (20) reports a most interesting series of investigations on pathologic cases made by the fractional method of gastric study with test breakfast and milk and egg diets, the samples being extracted by means of a long thin soft rubber Nélaton catheter. He shows trypsin to be present in 37 out of 61 cases and calls attention to regurgitation of duodenal contents as a normal process, but states that his results, not being uniform, do not justify an unqualified confirmation of Boldyreff's theory.

METHODS

The samples of gastric contents were obtained by the usual technique employed in this laboratory, namely, by fractional removal (21) through the Rehfuß tube (22). The experiments were all carried out on normal individuals whose last meal was that of the previous evening. The tube was introduced and the residuum carefully removed for study. Thereupon the material under investigation was introduced and samples of 5 cc. of gastric contents were removed for study at intervals of ten minutes; the experiments being continued until the stomach was empty and further specimens unobtainable.

Studies were made after the introduction of (1) acid solutions, HCl 0.542 and 0.4 per cent, vinegar (acetic acid 2.72 per cent); (2) water; (3) sodium bicarbonate solutions in various strengths 5 per cent, 2 per cent, 1 per cent, 0.65 per cent; (4) small Ewald meal—1 slice of toast and 240 cc. of water; (5) small Ewald meal with 240 cc. of a 1 per cent sodium bicarbonate solution instead of water.

The total acidity was determined by titrating 1 cc. of the sample of gastric contents against $\frac{N}{100}$ KOH, using phenolphthalein as an indicator, the values being expressed as the number of cubic centimeters of $\frac{N}{100}$ KOH necessary to neutralize 100 cc. of gastric contents. Free acidity was determined by the Sahli iodine method (23) and the values similarly expressed.

Alkalinity in certain specimens of a series was determined by titrating 1 cc. of the sample against $\frac{N}{100}$ H_2SO_4 using methyl orange as the indicator and the values expressed as the number of cubic centimeters of $\frac{N}{100}$ H_2SO_4 necessary to neutralize 100 cc. of gastric contents. Upon evidence of acidity in the series the total and free acid were estimated as above mentioned.

Tryptic estimations were made on samples immediately after removal by a method modified by one of us (24).

EXPERIMENTAL

The question of the residuum found in the fasting stomach has been studied most carefully in this laboratory (25, 26). Of thirty-four residua examined for trypsin only two failed to evidence its presence. Charts I and II show the tryptic values to be high in residua of low acidity and low in residua of high acidity. The tryptic values as

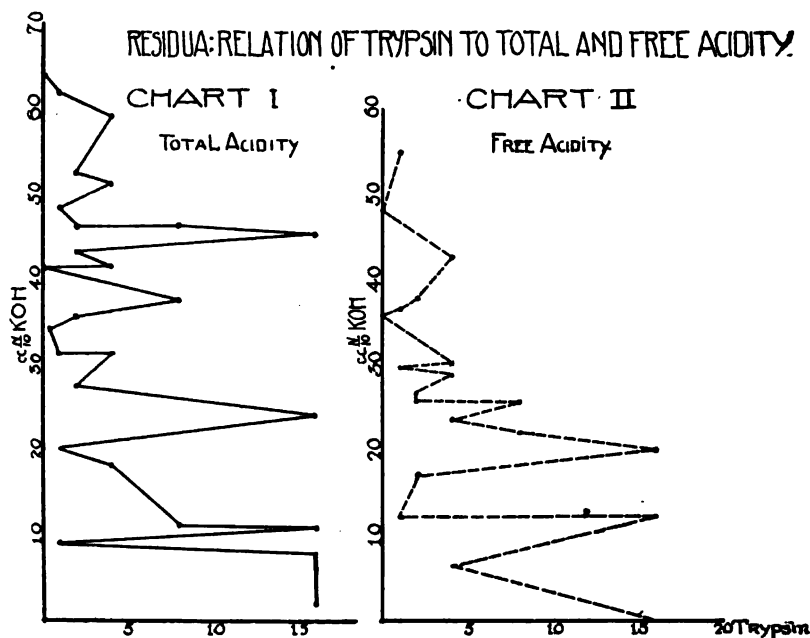
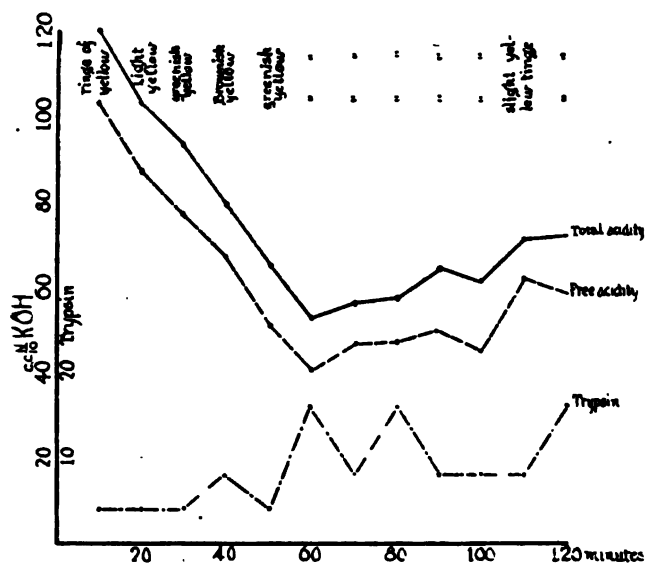


Fig. 1.

plotted in Chart II are shown to be almost inversely proportional to the free acidity. The residua were almost invariably highly colored.

From a total of over fifty experiments we have selected the following as typifying the results obtained after the introduction of the various materials mentioned.

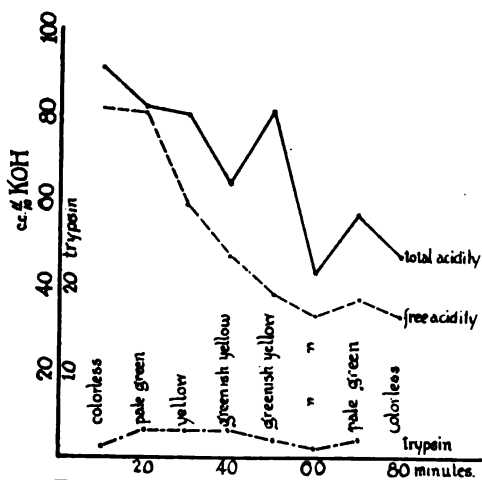
The influence of acid. Chart III is an experiment in which 100 cc. of 0.542 per cent HCl was introduced into the stomach. It is seen that the high acidity is rapidly reduced and with this reduction there is a coincident rise in tryptic values. Following the neutralization of the foreign acid to 0.2 per cent HCl a rise in acidity due to secondary



Diet: 100 cc. of 0.542% HCl at 23°C.

CHART III

Fig. 2.



Diet: 100 cc. of 0.4% HCl at 20.5°C.

CHART IV

Fig. 3.

stimulation of gastric secretion occurs. The first sample removed was slightly tinged with yellow and as the experiment proceeded the samples became a greenish and brownish yellow. On standing all the specimens assumed a dark green color. Chart IV is the curve of a similar experiment with the introduction of 100 cc. of 0.4 per cent HCl. The fall in acidity is accompanied by a rise in tryptic values, and a marked coloring of the samples substantiated regurgitation. The trypsin curve assumed low values, however. Other experiments with HCl and vine-

gar (2.72 per cent acetic acid) showed rapid reduction of acidity and corresponding evidences of regurgitation and neutralization. Toward the end of all the experiments the specimens became viscid in character.

The influence of water. Chart V shows a moderate stimulation of gastric secretion following the introduction of 100 cc. of water. The tryptic values are seen to be high, and it is interesting to note that the

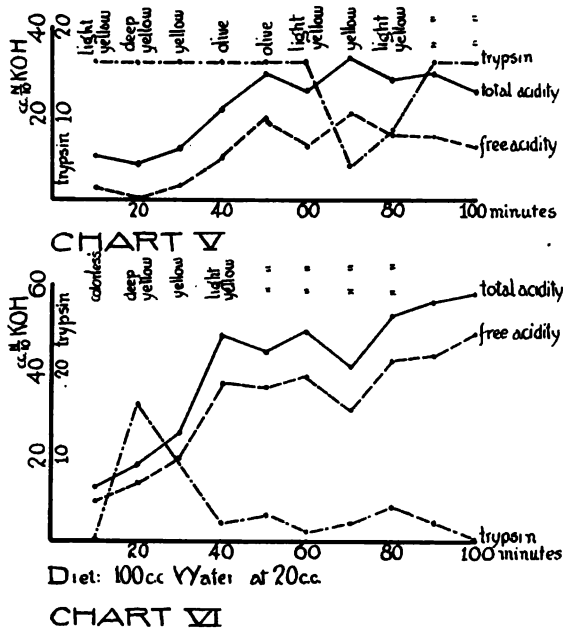


Fig. 4.

rather low curve of total acidity reaches its maximum only at that point where the trypsin curve shows its minimum value. This suggests a restraining influence on the acid rise exerted by the alkaline regurgitation in the early part of the experiment. The samples in this case were all highly colored. 100 cc. of water in the subject represented in Chart VI is followed by a more marked stimulation of gastric secretion. In contrast to the previous case the tryptic values run low, although trypsin is present in all specimens. Color changes here parallel the tryptic values.

The influence of a small Ewald meal. These tests were made with

one slice of toast and 240 cc. of water. The tube was introduced, residuum removed, the toast chewed finely and swallowed with the tube in situ, after the ingestion of the toast the liquid was introduced through the tube. Chart VII illustrates a response to the stimulation in a high acidity type of individual. Each specimen shows the presence of trypsin but the curve of the tryptic values takes a low level. No color changes were noted in the experiment. Chart VIII is the experiment repeated on an individual of the low acidity type. Trypsin

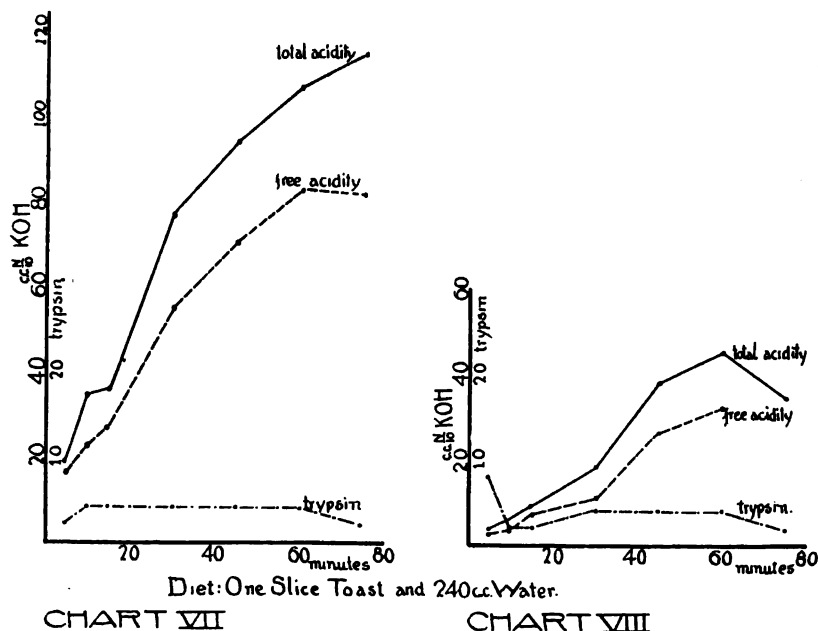
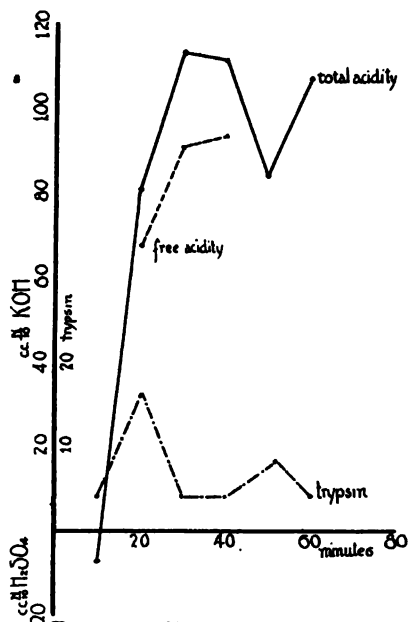


Fig. 5.

is seen to be constantly present, and, as in the previous case, no color changes in the samples resulted. It will be noted that 75 minutes were required for the stomach to empty itself in both of these cases.

The influence of a small Ewald meal with 240 cc. of 1 per cent sodium bicarbonate. Chart IX is an experiment on the same high acidity individual represented in Chart VII. The diet here consisted of one slice of toast but with 240 cc. of a 1 per cent sodium bicarbonate solution substituted for the 240 cc. of water of the previous experiment. The first ten-minute sample showed but slight alkalinity and in the following few minutes the gastric contents became acid, at the thirty-

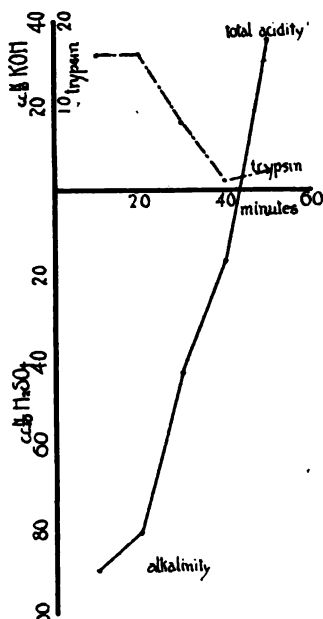
minute period reaching an acidity of 112 cc. $\frac{N}{10}$ KOH, or over 0.4 per cent HCl. There are two explanations for this fact; one, that an out-pouring of gastric juice neutralized the alkali present, the other that the greater portion of the alkaline fluid left the stomach and the remainder was neutralized. The latter seems the more probable, since to completely neutralize the 240 cc. of 1 per cent sodium bicarbonate solution would have required the secretion within a short period of



Diet: One Slice of Toast and 240 cc. of 1% NaHCO₃ Solution

CHART IX

Fig. 6.



Diet: One Slice of Toast and 240 cc. of 1% NaHCO₃ Solution

CHART X

Fig. 7.

200 cc. of gastric juice having an acidity of 0.5 per cent HCl. The fact that the stomach was empty in sixty minutes instead of seventy-five minutes, as with the same diet and water (Chart VII), also favors the latter explanation. This is not in accord with Cannon's theory (27) that the pylorus requires free acid on its gastric side for its opening, since material must have left the stomach while its contents were alkaline in reaction. The high acid values also speak against the theory that sodium bicarbonate inhibits the secretion of gastric juice as stated

by Cannon (28) and Pavlov (29). The presence of trypsin is to be noted in each sample although no evidence of color was found throughout the experiment. This experiment was repeated on the individual represented in Chart VIII. One slice of toast and 240 cc. of 1 per cent sodium bicarbonate solution were given (Chart X). A slower neutralization is shown than in the previous case, as would be expected from the low acidity type of individual (compare with Chart VIII). The same evidence of rapid evacuation is, however, present as in the previous case; the stomach emptying in fifty minutes with 1 per cent sodium bicarbonate solution and toast in contrast to seventy-five minutes with water and toast. It is evident that emptying must have been going on with the gastric contents alkaline in reaction. The tryptic values are high during the alkalinity, thus proving that regurgitation through an open pylorus had occurred. The fall in tryptic values suggests a closing of the pylorus produced either by a direct pyloric stimulus (30), or a duodenal reflex, due first to the coarse food particles and later to an outflow of acid into the duodenum. As in all our experiments with toast and water or alkalies, no color changes occurred in the specimens.

The influence of sodium bicarbonate. 100 cc. of a 5 per cent solution of sodium bicarbonate introduced into the stomach was followed in Chart XI by the neutralization and emptying of the gastric contents in less than forty minutes. A stimulation of gastric juice continues after this time and evidences the truth of the older views that alkalies have some stimulatory influence on gastric secretion. The tryptic values are seen to rise with the acidity and the color changes closely follow the increase in trypsin, suggesting a regurgitation to bring down the rather highly acid secretion to a non-irritating level. The introduction of 100 cc. of a 5 per cent sodium bicarbonate solution (Chart XII) in another case was followed by a slow emptying and a more gradual decline in the alkalinity to about that of the strength of the pancreatic juice. It is to be noted that the stomach emptied while its contents were still alkaline so that no acid was present to open the pylorus. The retention of the solution for ninety minutes indicates that the pylorus was tonic. The tryptic values remained constantly high and most of the specimens contained traces of bile, therefore, the pylorus must have opened intermittently to allow this regurgitation.

Charts XIII and XIV typify experiments in which 100 cc. of a 2 per cent solution of sodium bicarbonate were used in each case. Chart XIII shows rapid neutralization and emptying, followed by marked

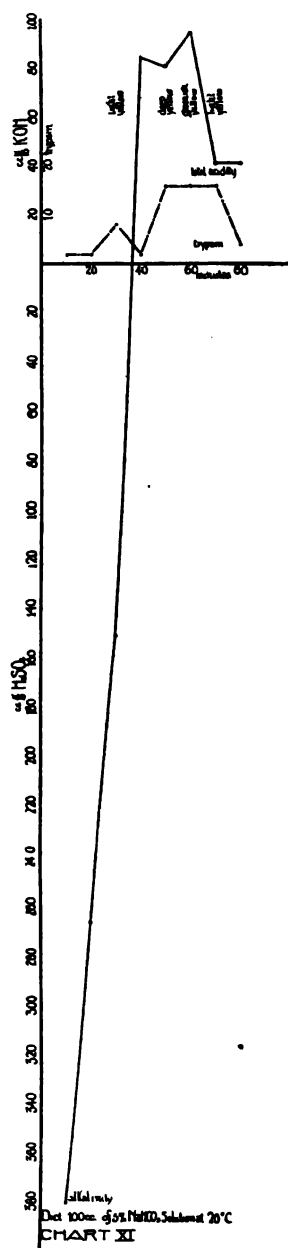


Fig. 8.

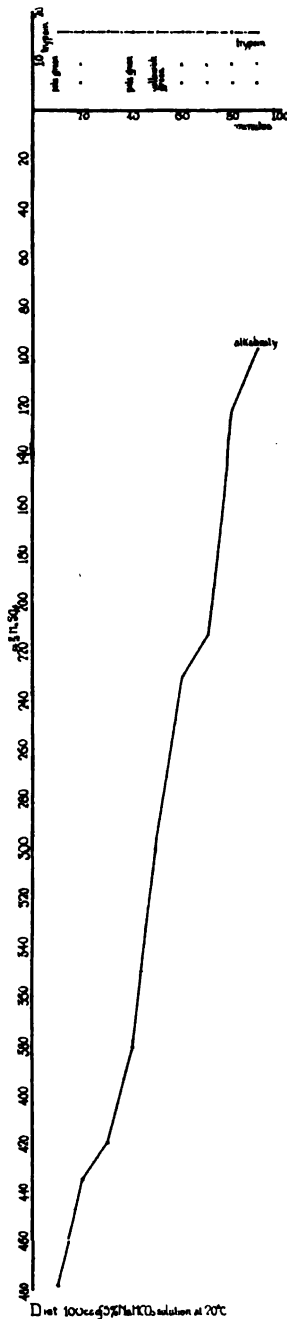


CHART XII

Fig. 9.

stimulation of gastric secretion. A rise in trypsin values and coincident color changes accompany the final fall in acidity. Chart XIV shows the first ten minute sample to be acid and a curious relation of trypsin and color to acidity. The fall in trypsin in the twenty-minute sample seems to allow a rise in acidity, while as the trypsin rises and the color deepens in the thirty-minute specimen the acidity is seen to fall.

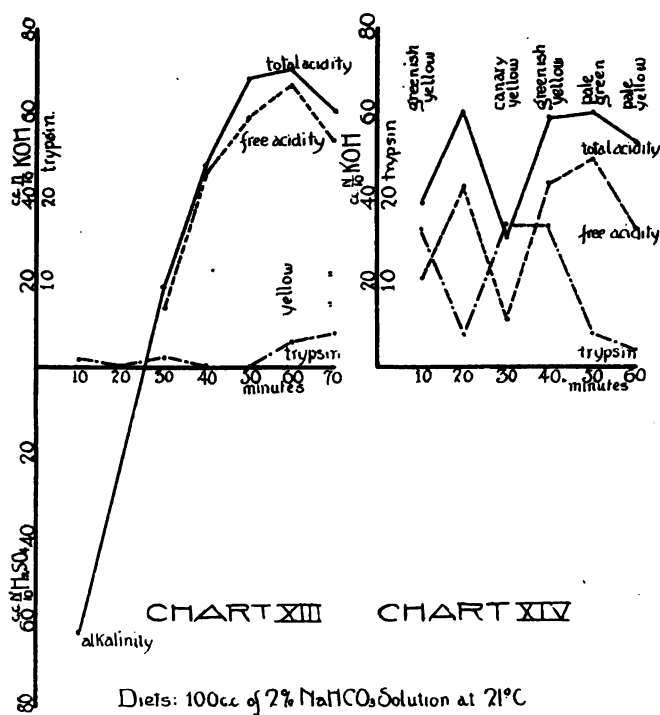
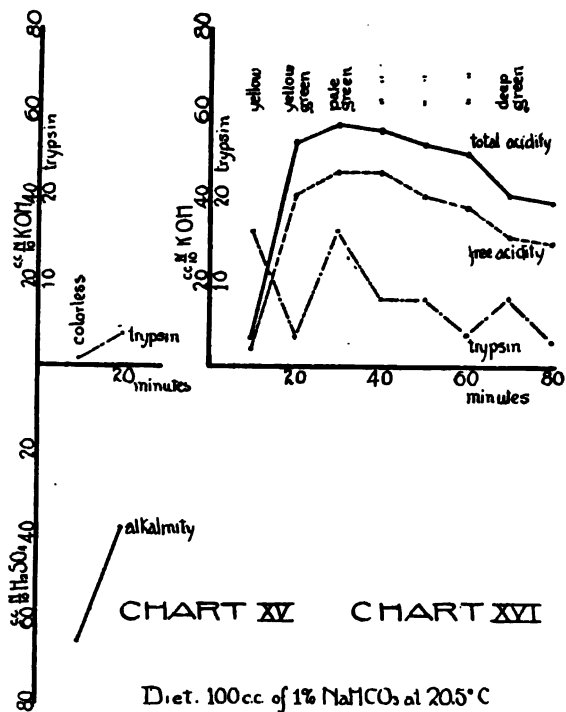


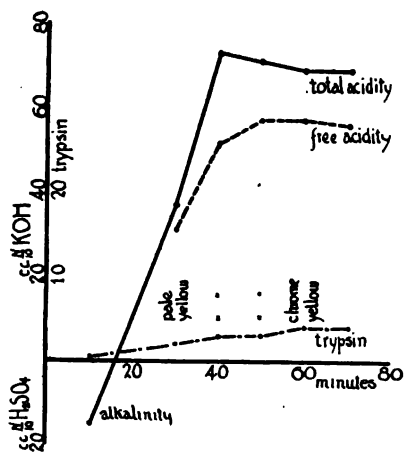
Fig. 10.

Charts XV and XVI are experiments in which 100 cc. of a 1 per cent sodium bicarbonate solution were introduced into the stomach. Chart XV shows rapid emptying of the stomach with alkaline reaction and no gastric secretory stimulation. Trypsin was present in both samples. Chart XVI shows rapid emptying and a marked stimulation of gastric secretion, the first sample being acid. The pronounced tryptic values and high color of the specimens indicate regurgitation of duodenal contents.



Diet: 100 cc. of 1% NaHCO_3 at 20.5° C

Fig. 11.



Diet: 100 cc. of 0.65% NaHCO_3 Solution, at 21.5° C.

CHART XVII

Fig. 12.

The introduction of 100 cc. of 0.65 per cent sodium bicarbonate solution, which is said to be the alkalinity of the pancreatic juice (31), was followed by pronounced stimulation of gastric secretion (Chart XVII). Tryptic values rise and the color of the samples deepen as the acidity increases, again suggesting a regurgitation of duodenal contents in an effort to check the mounting acidity.

DISCUSSION

One of the most striking facts to be observed in these experiments is the almost constant presence of trypsin in the fasting and digesting contents of the normal human stomach. The components of the pancreatic juice have been observed in the stomach of the dog by Pavlov, Schittenhelm (32) and Walter (33). Boldyreff (34) noted their presence when the gastric juice was highly acid. Volhard (35) and Lintvareff (36) found them when fats were in the stomach. Ehrenreich's previously mentioned treatise is the most complete series of the study of trypsin in the gastric contents of patients with gastro-intestinal disorders that we have found in the literature. He shows a tryptic enzyme to be present in a little over fifty per cent of his cases. Bickel (37) noted duodenal regurgitation in a girl with gastric and esophageal fistulae. Ehrmann and Lederer (38) have shown considerable regurgitation to occur in the usual Ewald-Boas test meal. Krivlov (39) reports that 63 per cent of 51 gastric analyses on 32 patients with gastro-intestinal disorders gave positive tests for trypsin. Azzovarisco (40) calls attention to the diagnostic value of trypsin in the gastric contents—it being absent in seven cases with disorders involving the pancreas and its ducts. We must assume from this evidence that the influx of duodenal juices into the stomach is a part of the normal sequence of digestive processes. Sokolov (41) observed that both pancreatic juice and bile when introduced into the larger stomach of Pavlov dogs caused a secretion of gastric juice in the smaller pouch.

It was noted throughout our experiments that normal individuals of the high acidity type usually yielded low trypsin values, while in those of the low acidity type the tryptic power was marked. This is best illustrated by the composite Charts I and II, for the acidity of the residua has been found in this laboratory (42) to be an index of the type of secretion poured out during digestion. This suggested to us the possibility of tryptic digestion occurring in part in the stomach as a sort of compensatory action in cases of low acid and pepsin secretion.

Chittenden and Cummins (43) state that trypsin is active in a neutral or weak combined acid medium.

The introduction of acids into the stomach is followed by a rapid reduction of acidity to about 0.2 per cent HCl or less. The fall of acidity is accompanied by a rise in tryptic values and the presence of bile. The relation between trypsin and acid values may be assumed to be analogous to that found in hyperacidity cases and like them usually shows the fluctuation of trypsin at low values. These low trypsin values may be explained by the character of pancreatic juice, as produced by an acid stimulus: for Pavlov (44) has shown that while the juice secreted under the influence of nerve stimulation is rich in enzymes and protein but poor in alkalies, that produced by an acid stimulus contains relatively little enzymic power or protein but is rich in alkali.

It has been demonstrated (45) that trypsin will adsorb to specific and non-specific substrata, and that this adsorption is dependent upon the H-ion concentration of the solution. When true aqueous solutions or water alone are introduced into the stomach, the low tryptic values with high acidity can hardly be due to adsorption of some of the trypsin, although with the test meal such adsorption is probable.

With regard to the action of the HCl and pepsin of the gastric juice on trypsin. Our experiments were, of course, conducted on freshly removed samples, but we have found trypsin present in samples having an acidity of 110 cc. $\frac{N}{10}$ KOH, which had stood for eighteen hours at room temperature. Other tests have shown that trypsin seems but little influenced by the acid and pepsin in the gastric contents. Long and Muhlman (46), working with artificial laboratory pancreatic products, found that trypsin would withstand an acidity of 0.3 per cent HCl through thirty minutes at 40° C., but the destructive action of HCl on trypsin was much accelerated by the presence of pepsin. Ehrmann and Lederer (47) find trypsin very resistant to the action of the gastric juice. This was confirmed by Ehrenreich (48).

Five per cent sodium bicarbonate solutions, in those cases in which a prompt response of gastric secretion did not result, are seen to be held in the stomach for a considerable period, with marked color changes and high trypsin values in the abstracted samples. This retention seems to be for the purpose of allowing the reduction of the high alkalinity by the acid of the gastric juice. The high trypsin values and color changes may be explained by antiperistalsis and regurgitation, the result of the irritation of the duodenum by the highly alkaline fluid. This irritation in the duodenum also produces retention by closing the

pylorus. In those cases in which the alkaline fluid was of low percentage, or the response of the acid gastric secretion and consequent neutralization to a non-irritating point was prompt, the material quickly left the stomach, and, the rapid flow being directed toward the duodenum, regurgitation was slight and trypsin values consequently low.

It is obvious from the sodium bicarbonate studies that the presence of acidity on the gastric side is not necessary for the opening of the pylorus, as stated by Cannon (49). From our observations we have been led to concur with the European view—that the pylorus is controlled reflexly from the duodenum (50). Acid from the stomach irritates the duodenal mucosa and the pylorus becomes temporarily firmly closed. As this acid is neutralized by the alkaline secretions of the duodenum—the pancreatic juice, bile and succus entericus—the pylorus relaxes and more of the gastric contents are forced into the duodenum. This intermittent opening and closing continues until the stomach is empty (51, 52). Fats as contained in milk and egg white when present in the duodenum are known to close the pylorus (53, 54). Distention of the duodenum also reflexly closes the pylorus (55, 56, 57). Coarse food acting on its gastric side will close the pylorus (58). Liquids at high or low temperatures close the pylorus and are retained in the stomach until brought to body temperature (59). It is claimed that hypotonic and hypertonic solutions also leave the stomach more slowly than isotonic solutions (60, 61).

The evidence seems to demonstrate that materials which are irritating to the duodenum are retained in the stomach in an effort to render them non-injurious to the small intestines. Strong sodium bicarbonate solutions are held until the acid secretion of the stomach reduces their alkalinity to approximately that of the duodenum. Acids in the stomach are partially neutralized by regurgitation of alkaline duodenal contents before they leave the stomach. Non-irritating materials as weak sodium bicarbonate solutions or water leave the stomach rapidly because they do not excite the duodenal reflex necessary to produce the pyloric closure.

We desire to call attention to the stimulatory effect on human gastric secretion of sodium bicarbonate solutions. This fact was first set forth by Claude Bernard (62) and later corroborated by Jaworski (63), Zasadki (64), Du Mesnil (75), Lenoissier and Lemoine (66) and others. They appear to act differently in animals, for in Cannon's experiments (67) on cats, he noted an inhibition of gastric secretion and a delayed

emptying of the stomach following the mixing of food with 1 per cent sodium bicarbonate solution. Pavlov (68) also states that solutions of sodium bicarbonate varying from 0.05 to 1 per cent inhibited the secretion of gastric juice in dogs. In our experiments on normal human adults we find the reverse to obtain—a marked stimulation of acidity in most cases and evidences of material leaving the stomach during the period in which its contents are alkaline in reaction, together with a shortening of the time required for complete emptying of the stomach. It appears, therefore, that the beneficial results ensuing from the use of weak sodium bicarbonate solutions in gastric disorders are not alone due to neutralization of excessive acidity, but perhaps more to the fact that they more rapidly empty the stomach, preventing stasis and thus shortening the period of work for the stomach and increasing the length of its periods of rest. Reichmann (69) has shown that sodium bicarbonate has no permanent effect on gastric secretion and ascribed its beneficent action wholly to its power to neutralize the acidity in the stomach.

The presence of bile coloring in the samples, in those experiments in which the diet consisted of substances stimulating biliary secretion, bears a close relation to the tryptic values. In general those samples most deeply colored have high tryptic content. This, however, is not the invariable rule.

The previously discussed variation of the quantity of trypsin in the pancreatic juice, depending on whether produced by nerve or acid stimulus renders trypsin not an infallible indicator of the amount of regurgitation. A comparatively small amount of nerve excited secretion may contain a large amount of the enzyme, while a greater quantity of the thin watery secretion of higher alkalinity caused by acid stimulation may contain but little enzymic power. Still the fact that it is always present renders its estimation the best method at hand for the determination of duodenal regurgitation.

Our work in many ways confirms the theory of Boldyreff "The self-regulation of the acidity of the stomach," in that we find regurgitation of duodenal contents a constant factor in the workings of the normal human stomach. The evidence seems to clearly show that the function of the gastro-duodenal portion of the alimentary tract is to so prepare ingested materials that they shall be non-irritating to and best adapted for absorption by the small intestines. Regurgitation of duodenal contents into the stomach is a response to irritation of the duodenum and part of an attempt to render harmless substances that would have an

injurious effect on the small intestines. This phenomenon occurs not only with high acidity but when the gastric contents are alkaline in reaction and seems to be a constant accompaniment of normal gastric digestion.

CONCLUSIONS

1. A tryptic enzyme is almost constantly present in the fasting and digesting contents of the normal human stomach.
2. The tryptic enzyme is deduced to be trypsin regurgitated from the duodenum.
3. Trypsin in the gastric contents is highly resistant to the action of acid and pepsin.
4. In general—the tryptic value is high in the presence of low acidity and in alkaline reaction, and of low value when the gastric contents are of high acid concentration. A fall in the acidity is usually accompanied by a rise in the tryptic values.
5. The color of the gastric contents often changes during the period of experiment from that of the ingested material to a golden yellow or a dark olive or blue green. This color change is due to regurgitation of bile from the duodenum and is absent on a diet of substances which do not cause the outpouring of bile.
6. The tryptic values in the gastric contents usually rise concomitantly with the color change, although in a non-bile stimulating diet the tryptic value seems independent of the color.
7. Sodium bicarbonate in 5 per cent solution is held in the stomach until sufficient HCl is secreted to bring the alkalinity to a point where it is non-irritating to the duodenum. The retention is accompanied by a high trypsin value—suggesting anti-peristalsis in the duodenum in response to an irritant.
8. Sodium bicarbonate in 1 per cent solution hastens the emptying of the stomach either by increasing the motility of the stomach or opening the pylorus.
9. Sodium bicarbonate solutions do not inhibit human gastric secretion, but seem to have a direct stimulatory effect in some cases.
10. Free HCl seems unnecessary for the opening of the pylorus—for the stomach sometimes empties while its contents are still alkaline.
11. 0.5 per cent HCl ingestion is followed by a rapid fall in acidity to about 0.2 per cent, due to a regurgitation of alkaline duodenal contents, as is indicated by the rise in tryptic values coincident with the fall of the acidity. The acid is then emptied from the stomach.

12. Regurgitation of duodenal contents into the stomach is but another of the protective functions of which the body furnishes so many examples and has for its purpose the defense of the small intestines from irritants.

POSTSCRIPT

After this paper was ready for the press the work of Zaitzeff (Russkiy Vrach, 14, no. 29, 1915) came to our notice. In experiments on five dogs with duodenal or intestinal fistulas he demonstrated the regurgitation of pancreatic juice into the stomach and showed trypsin to be present in the stomach contents.

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LIVER CIRCULATION IN RELATION TO GLYCEMIA

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While many investigations of liver anemia and circulatory changes have been made, the reports are discordant and widely scattered in the literature. The relative importance of the portal and hepatic circulations to glycemia has been but little emphasized. We (1) have recently attributed peptone hypoglycemia to such changes and therefore think it worth while to restate the investigations on liver circulation and to add some new observations, insofar as they relate to glycemia, under the following headings: Portal obstruction or ligation, Hepatic ligation, Anemia due to the simultaneous interruption of both circulations, Hyperarterialisation and changes due to increased venous blood flow through the organ (reversed Eck fistula).

PORTAL LIGATION

Bernard (2) devised a method which caused slow obliteration of the portal vein and found that the operated animals developed an alimentary glycosuria. Burjenko (3) obliterated the portal vein in 35 animals and observed them from one to 14 months afterward without finding glycosuria. The blood sugar was not determined. Allen (4) could not confirm Bernard's findings and suggests that the facts obtained by Bernard may have been due to indirect injury of the pancreas and not to portal ligation *per se*. In support of this view Gilbert and Chabrol (5), found chronic pancreatitis which involved the islets following such operations. Natus (6) also found inflammatory changes in the pancreas following portal stasis and internists (7) believe that portal stasis and disturbance of the liver circulation may be the cause of inflammatory changes in the pancreas sufficient to cause glycosuria. To avoid the stasis and consequent changes in the pancreas and other organs which follow ligation of the portal vein, various investigators made Eck fistulas and studied the effects. Hedon (8) worked with depancreatized animals and claims to have obtained different results

when the blood of a normal animal was transfused into the portal circulation of the depancreatized animal than when it entered the general circulation before passing through the liver. According to Hedon the internal secretion of the pancreas, to be effective, must enter the portal circulation. If this be true the Eck fistula should produce glycosuria. It is generally held however that it does not and in support of this general opinion Carlson and Drennan (9), also Carlson and Ginsburg (9), found that the glycosuria and the hyperglycemia that follows removal of the pancreas can be prevented in pregnant animals by the internal pancreatic secretion of the young in utero, which does not enter the general circulation through the portal system. Forschbach (10) also showed that pancreatic glycosurias may be prevented by parabiosis, and various authors have shown that pancreatic grafts may prevent or lessen the glycosuria. In none of these cases does the internal secretion of the functioning pancreas enter the general circulation through the portal vein and liver.

De Filippi (11), Hawk (12) and Macleod (13) have studied carbohydrate metabolism after Eck fistulas and have never reported glycosuria. Michaud (14) found that Eck fistula prevented adrenalin glycosuria but when the dog received 100 grams of dextrose per os the blood sugar reacted as in the normal animal, i.e., remains normal or increases within normal limits. Macleod (*loc. cit.*, xxii) also found that clamping the portal for short periods of time did not produce glycosuria. It is in such short periods of time that we should expect changes in the concentration of the blood sugar if glycosuria occurs. In slow obliteration of the vessel, or in operations where the animal recovers, the body in all probability would rapidly adjust itself to such changes. If however the blood circulation through the liver is quickly changed, it should be possible to detect changes in the sugar content in the blood in an hour or less. Such changes may not be seen if we rely on glycosuria as the test because the degree of glycemia must be marked before glycosuria appears. The absence of sugar in the urine therefore does not indicate the absence of glyceemic changes.

To test this point we selected a number of healthy dogs. Each was anesthetized with ether, a tracheal cannula inserted and also a cannula in one of the carotids from which to obtain the samples of blood for analysis. The ether was kept constant throughout the experiment. Blood was taken immediately before ligation of the portal and again about an hour afterward. The sugar was determined by the Bertrand method. The results are given in Table I.

TABLE I

Result of ligation of the portal vein on the blood sugar

DOG NO.	PER CENT OF DEXTROSE IN BLOOD		TIME AFTER LIGATION IN MINUTES	INCREASE ACTUAL
	Before ligation	After ligation		
2	0.088	0.153	60	0.065
3	0.054	0.105	60	0.051
5	0.066	0.127	75	0.061
6	0.123	0.192	65	0.069
27	0.080	0.146	55	0.066
28	0.084	0.314	60	0.023
29	0.102	0.128	65	0.026
30	0.116	0.167	32	0.051
Average.....	0.089	0.145	59	0.056

LIGATION OF THE HEPATIC ARTERY

Arthaud and Butte (15) after ligation of the hepatic artery found first-hyperglycemia, probably due to the struggling of the animal or to hemorrhage. They took 100 grams of blood for sugar determination. After a transient hyperglycemia a state of hypoglycemia followed. Tangl and Harley (16) after ligation of the abdominal arteries allowed the animals to come out of the anesthesia and found hypoglycemia. However the animals lived only 5 to 7 hours and were undoubtedly

TABLE II

Results of ligation of the hepatic artery

DOG NO.	RESULTS OF LIGATION OF THE HEPATIC ARTERY		TIME AFTER WHEN DETERMINED	CHANGE IN DEXTROSE
	Sugar before operation	Sugar after operation		
1	0.080	0.130	60	0.050
4	0.116	0.178	60	0.062
8	0.120	0.140	60	0.020
33	0.065	0.080	60	0.015
34	0.095	0.150	60	0.055
35	0.087	0.092	60	0.005
9	0.126	0.113	60	-0.013
26	0.148	0.124	60	-0.043
Average.....	0.105	0.124	60	0.019

shocked and moribund. The results are therefore of doubtful value. In keeping with their results Allen (17) found that ligation of the hepatic artery does not lower the dextrose tolerance and does not render the animal more susceptible to diabetes nor does the ligation itself cause diabetes. Piqure was still effective after ligation of either the hepatic artery or the portal vein, but it has been known from the time of Bernard that the simultaneous ligation of both prevents it.

To obtain further data on the immediate effects of hepatic ligation a number of experiments were carried out in the same manner as that described for portal ligation. The results are given in Table II.

LIGATION OF BOTH HEPATIC AND PORTAL AND COMPLETE REMOVAL OF THE LIVER

Bock and Hoffman (18) worked with rabbits and found that after ligation of both portal and hepatic circulations the blood sugar entirely disappeared (quoted from Tangl and Harley) Seegen (19) ligated the vena cava and the aorta above the diaphragm and kept the animals living with artificial respiration. The sugar content of the blood was reduced in three experiments (I) from 0.146 per cent to 0.04 per cent in 70 minutes and (II) from 0.136 per cent to 0.067 per cent in 36 minutes and (III) from 0.230 per cent to 0.120 per cent in 60 minutes.

Kaufmann (20) confirmed Seegen's results. Minkowski (21) removed the liver from geese and found that the sugar disappeared from the blood. Schenck (22) and Kausch (23) confirmed the work of Bock and Hoffman, and Minkowski, at least to the extent that the blood sugar is much reduced when the liver vessels are ligated. Pavy and Siau (24) studied the effects of liver ligation and removal and concluded that while ablation of the liver causes a fall of the blood sugar the lowest figure they obtained was 0.044 per cent. They never found the blood free from sugar, hence could not entirely confirm the work of Bock and Hoffmann. Further, although they report only two experiments, ligation of both hepatic and portal did not lower the sugar concentration but instead at the end of their experiments the sugar was 0.152 per cent and 0.254 per cent, or more than is normally present. They state that with the exception of Tangl and Harley, other experimenters have found that without the removal of the liver, or blockage of the cava above it, no fall of the sugar concentration of the blood takes place. They think that ligation below the liver may still allow sugar to pass from the liver to the blood.

We made a number of experiments on the ligation of both portal and hepatic vessels and obtained the results given in Table III. The experiments were carried out in the same way as the preceding:

TABLE III
Portal and hepatic ligation

DOG NO.	PER CENT DEXTROSE BEFORE OPERATION	DEXTROSE AFTER	TIME AFTER	INCREASE OR DECREASE
37	0.057	0.054	60	-0.003
1	0.130	0.180	45	0.050
2	0.153	0.136	75	-0.014
4	0.178	0.170	60	-0.008
5	0.127	0.083	30	-0.044
6	0.173	0.126	30	-0.047
7	0.077	0.090	45	0.013
9	0.113	0.076	35	-0.043
Average.....	0.126	0.114		-0.012

HYPERAEMIA

Bernard (25) refers to the belief of Pavy that arterialisation of the blood flow through the liver suffices to cause glycosuria. This agrees with the clinical observation that the livers of many diabetics are more or less hyperemic. Jardelet and Niviere (26) state that the direct transfusion of the arterial blood of a rabbit into the mesenteric vein of another gives rise to glycosuria. Lepine (27) however could not confirm this assertion. Arthaud and Butte (28) observed glycosuria after ligation of the splenic and right gastro-epiploic arteries, and believe that this glycosuria is due to an increased blood flow through the liver caused by such ligation. Schiff (29) claims that ligation of the afferent renal veins in the frog, by increasing the blood flow through the liver, produces glycosuria. Langendorff (30) failed to confirm this work. Allen (31) studied the influence of varying the blood flow through the liver and concludes that ligation of the hepatic artery does not render the animal more susceptible to diabetes which one might expect if hypoarterialisation was an important factor in diabetes. In the attempt to increase the blood flow through the liver he anastomosed the splenic artery and vein and removed the spleen. He found this did not cause diabetes. The blood was not examined. There was some polyuria which he thinks was nervous, but at the same time the polyuria without glycosuria might be explained by a sudden loss of the glycogen of the liver. The glycogen content of the liver was not examined to decide

this suggestion. In support of the idea, however, it is known that hyperglycemic states may exhaust the glycogen and end in hypoglycemic states. In keeping with this statement, Lepine (32) says that ligation of the hepatic artery is followed in a few hours by a total disappearance of the liver glycogen. Our results do not suggest such a possibility.

Hyperarterialisation of the liver was obtained by two methods: One by turning the aorta into the portal directly by means of a glass cannula. Second: The vena cava and portal were anastomosed as for an Eck fistula, but instead of ligating the portal the vena cava was tied above the anastomosis—Reversed Eck. The aorta was then turned into the vena cava below the anastomosis. The starred numbers in Table IV were obtained by this technique.

TABLE IV
Hyperarterialisation of the liver

DOG NO.	PER CENT OF BLOOD SUGAR		TIME AFTER WHEN BLOOD ANALYZED	CHANGE IN AMOUNT SUGAR
	Before operation	After		
13	0.064	0.050	45	-0.010
14	0.165	0.186	51	0.021
15	0.156	0.200	15	0.044
16	0.149	0.313	68	0.164
17	0.206	0.465	68	0.259
18*	0.122	0.137	61	0.015
19*	0.306	0.330	20	0.024
12*	0.241	0.220	31	-0.021
Average.....	0.176	0.238		0.062

Hypervenosity was obtained by the reversed Eck fistula alone. The results are given in Table V.

TABLE V
Hypervenosity of the liver

DOG NO.	PER CENT OF BLOOD SUGAR		TIME AFTER WHEN BLOOD ANALYZED	CHANGE IN AMOUNT SUGAR
	Before operation	After		
20	0.162	0.150	37	-0.012
22	0.167	0.136	65	-0.031
23	0.072	0.123	85	0.051
24	0.163	0.164	45	0.001
36	0.142	0.214	60	0.072
Average.....	0.141	0.157		0.016

From our experiments it would seem that ligation of portal and hyperarterialisation are the only means to raise the blood sugar. The other changes are so small as to be attributed to the ether and within the limits of error.

DISCUSSION

In the present work we have confined our investigations to acute changes in the liver circulation. We have done so because acute changes are more likely than chronic changes to influence the blood sugar. The rather rapid adaptive powers of the body tend to obscure the influence of chronic changes and while there may be a direct action on the liver that entails marked changes in the blood sugar, the compensatory changes may conceal them. Again, disturbed liver circulation may cause secondary changes in the pancreas and these in turn may cause marked changes in the blood sugar and so obscure entirely any change due solely to the direct action on the liver.

In the acute changes, however, we are not entirely free from obscuring secondary influences. For: If an anesthetic be used, it, *per se* changes the sugar concentration of the blood. If we decerebrate the animal we can not avoid the difficulty. We have found the sugar change due to decerebration is just as great as that due to ether, and Morita (33) has recently shown that in decerebrate rabbits which were allowed to recover, ether, diuretin pain, etc., cause just as great a change in the blood sugar as in the normal animal. If we worked without any of these procedures the pain and shock produced would cause still greater changes. Again the changes in other organs must to some degree also enter into the mechanism of acute cases. Such changes as congestion of the other abdominal organs, and the extra work on the heart that many operative processes involve, can not be without effect.

One of the most difficult questions of the problem to answer satisfactorily is: Whether after an Eck fistula or its reverse, we get the actual increase of the venous blood flowing in the changed direction that the theory demands. In this region as is well known, the venous pressure is very low, consequently the mechanical obstructions which must be introduced by the roughened edges of the operated vessels, and the fall in the general blood pressure may so lessen the blood current that even less venous blood passes through the new route than before the establishment of the reversed Eck fistula. Again after ligation of the hepatic artery the result may be greater than the actual lessening of the arterial flow, because it seems to us that one of the functions of this

artery is to aid mechanically in advancing the low pressure venous blood through the liver to the heart, also following the ligation of the hepatic artery relatively more venous blood probably returns through the portal system. We can devise no method which will eliminate these possible objections, and where so many variables exist it is clearly impossible to state in mathematical terms the changes in the blood sugar which the modifications of the circulation in each artery or vein may cause.

The tables show that ligation of the portal vein caused a rise in the blood sugar of 0.056 per cent or about 62 per cent of the original value. This may be due mainly to asphyxia although the hepatic artery still carries oxygenated blood to the liver. We are inclined to believe from the slight effect of ligation of this latter vessel that its function is mainly mechanical as stated above and that the portal vein is relatively much more important for all functions of the liver. Hepatic ligation caused a rise in the blood sugar of 0.019 per cent or 18 per cent of the blood sugar before operation—about the same or less change than the ether alone would have caused. This is in keeping with the fact that the hepatic artery may be permanently ligated without noticeable change in the welfare of the animal. Ligation of both portal and hepatic caused an increase of 0.012 per cent or 9 per cent of the original sugar, which is less change than the ether alone might have caused. The results of this are also in agreement with the statements of Pavy (34) and Siau. Hyperarterialisation increased the blood sugar 0.062 per cent or 35 per cent of the original content. The mechanism here is probably a flushing out of the sugar or glycogen content of the liver and partly asphyxial due to pressure. The livers in these cases become swollen though not so markedly as might be expected. Hypervenosity caused a change 0.016 per cent or 11 per cent—a figure which ether alone might readily produce. In recent work with ether anesthesia we found (35) that the average increase of the blood sugar in one hour due to ether alone ranged from 5 to 26 per cent of the original concentration. Two only of the modifications of the liver circulation produce noticeable changes in the blood sugar, and these were not sufficient to cause glycosuria. These were ligation of the portal vein and the direct turning of the aortic blood through the liver. Less radical changes were without important effects. It is perfectly obvious therefore that the greatest conceivable uncomplicated changes in the liver circulation can play but a very unimportant part in glycosuria or diabetes.

The changes produced by any of the methods used are not great.

Ligation of the portal vein causes an increase in the blood sugar while ligation of the hepatic artery causes no increase. Several possible explanations are suggested. First, ligation of the portal and the prevention of its blood from passing through the liver causes an accumulation of dextrose from the gastro-intestinal tract simply because it is not removed by the liver. This explanation is not tenable because the simultaneous ligation of the hepatic artery and portal vein causes a decrease which would not be possible if sugar was accumulating in the venous system from the gastro-intestinal tract.

A second explanation: The liver for some unknown reason does not take up dextrose readily from the arterial blood.

Third: Arterial blood takes up glycogen from the liver more readily than venous blood. Hyperarterialisation therefore causes an hyperglycemia while hypervenosity does not. These facts and the lack of hyperglycemia following hepatic ligation leads us to think that venous blood is not capable of getting through the liver with as much dextrose as arterial blood. This may be due to the venous blood lacking any considerable power to take up dextrose from liver glycogen. This has an important bearing on the opinion held by many that asphyxia is an important factor in causing glycosuria. Could not the hyperglycemia be the result of a reaction to asphyxia, i.e., hyperoxygenation of the blood and this hyperoxygenation be the cause of the glycemia? This would be in harmony with the theory of Henderson and Underhill (36).

From Macleod's (37) work on the influence of CO_2 on glycogenolysis we might expect a greater action *ceteris paribus*—from the venous blood than the arterial. The action of CO_2 however can not be considered specific since Phloridzin has the same effect (38). We have found no change in the diastatic action of the blood caused by the various operative procedures, and do not therefore consider any of the changes due to enzyme action.

Summary Table

	INCREASE CALCULATED BY DIFFERENCE IN PER CENT BEFORE AND AFTER OPERATION	INCREASE EXPRESSED IN PER CENT OF SUGAR CONTENT BEFORE OPERATION
Ligated portal.....	0.056	62
Ligated hepatic.....	0.019	18
Portal and hepatic.....	0.012	9
Hyperarterialisation.....	0.062	35
Hypervenosity.....	0.016	11

CONCLUSIONS

1. Ligation of the portal vein caused a considerable hyperglycemia.
2. Ligation of the hepatic artery causes no hyperglycemia.
3. Simultaneous ligation of the portal vein and hepatic artery causes no hyperglycemia.
4. Hypervenosity of the liver causes no hyperglycemia.
5. Hyperarterialisation of the liver causes a significant hyperglycemia.
6. The utmost conceivable uncomplicated change in the circulation of the liver can play but a minor rôle in the production of glycosuria or diabetes.

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CORRECTION

In the December, 1915, issue of this Journal (vol. xxxix, no. 2) a number of the figures accompanying the article by Forbes and Gregg entitled: "Electrical Studies in Mammalian Reflexes. II. The Correlation Between Strength of Stimuli and the Direct and Reflex Nerve Response," were, through a misunderstanding, printed on the wrong kind of paper and failed to show many details which appeared in the original photographs. These figures are, therefore, reproduced for reference.

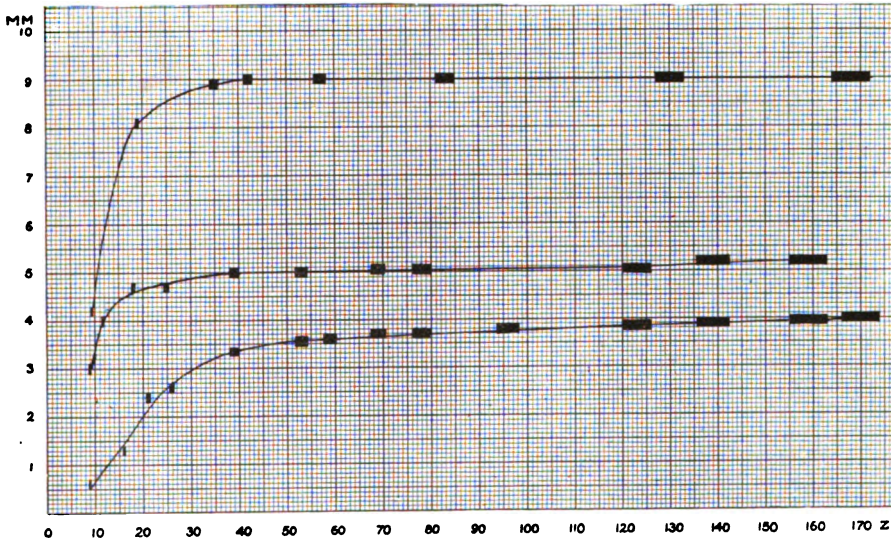


Fig. 1

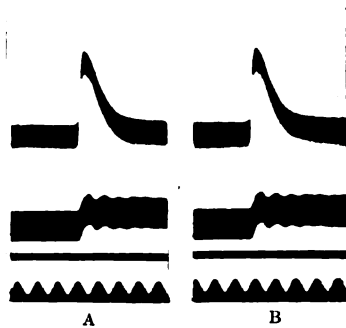


Fig. 2

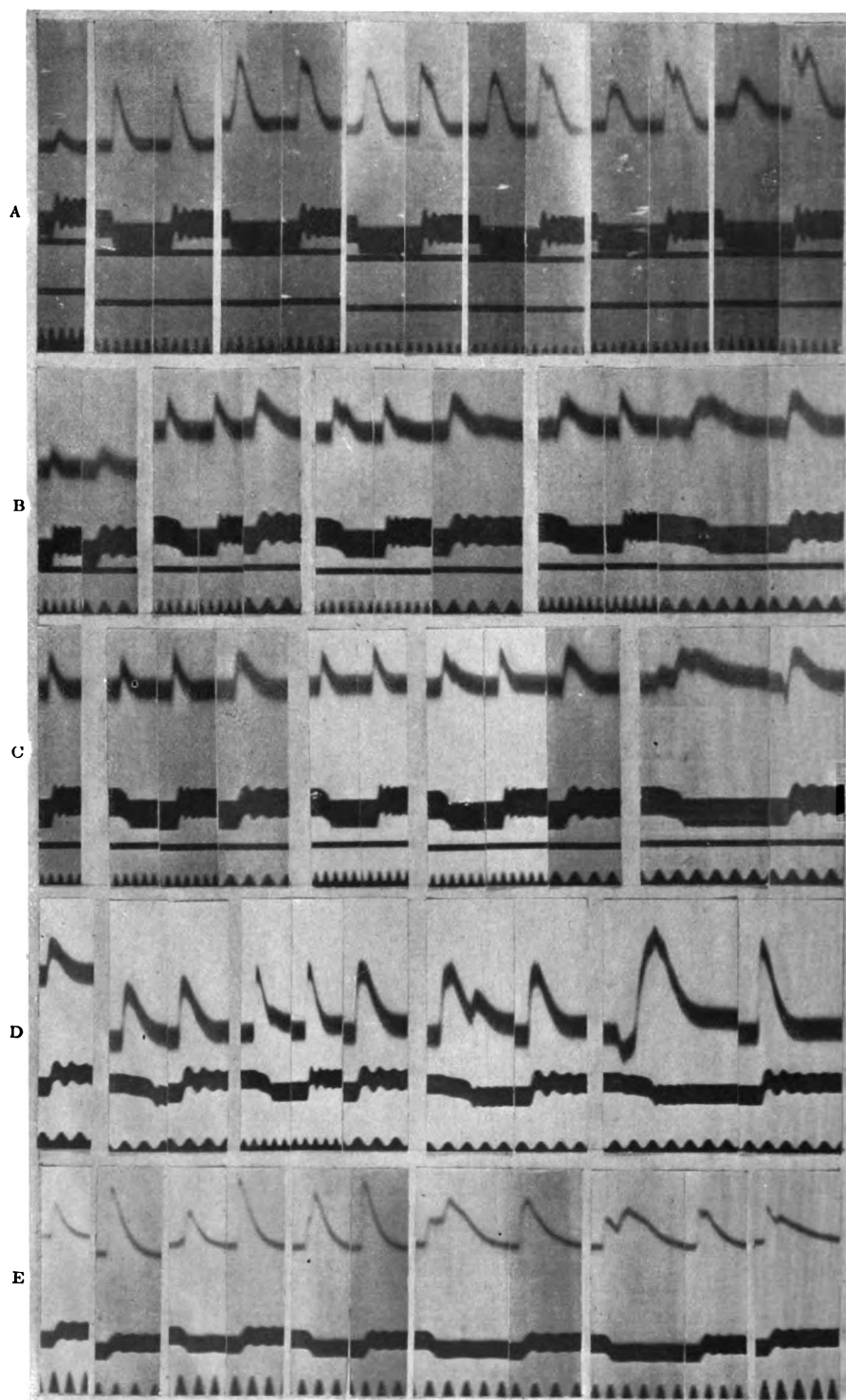


Fig. 3

ELECTRICAL STUDIES IN MAMMALIAN REFLEXES

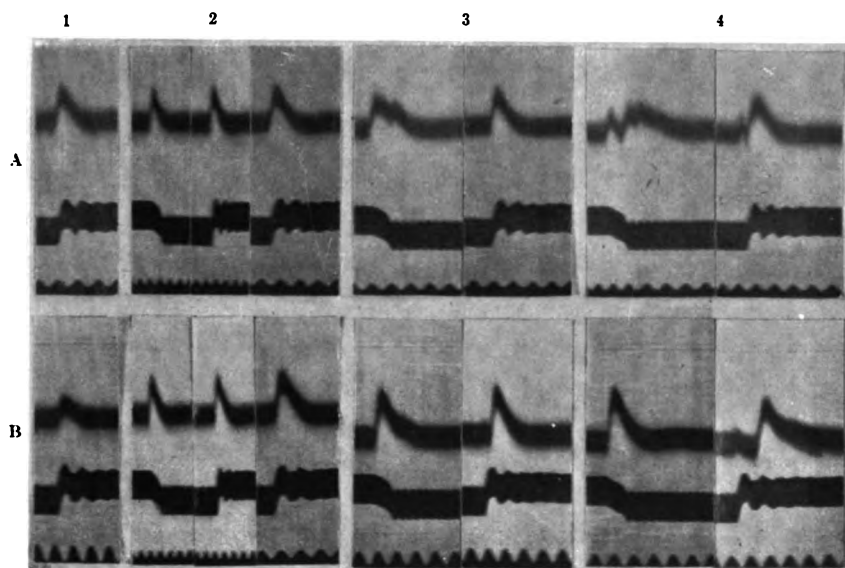


Fig. 4

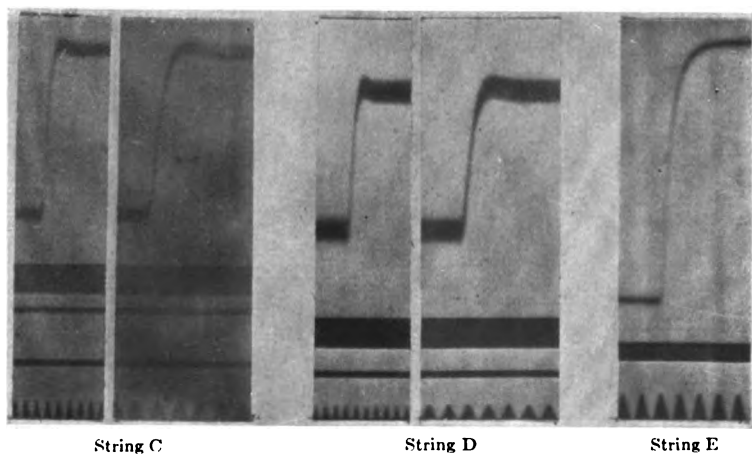


Fig. 5

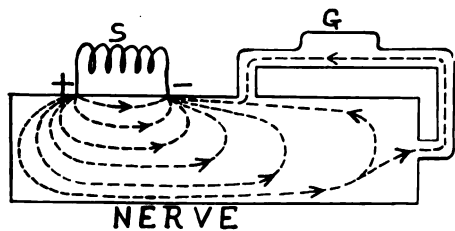


Fig. 6

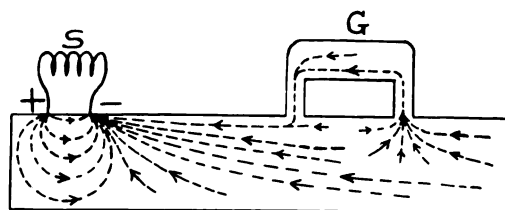


Fig. 7

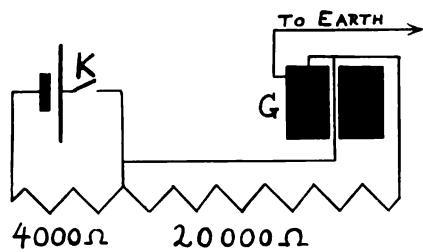


Fig. 8

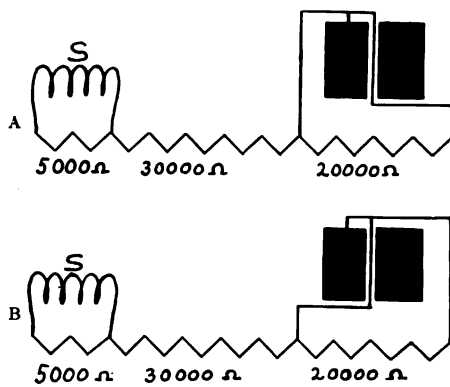


Fig. 10

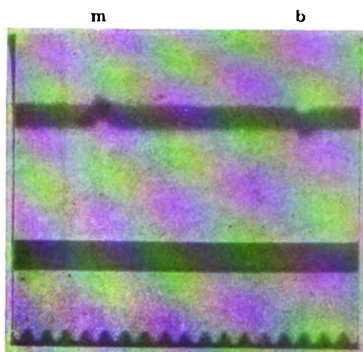


Fig. 9

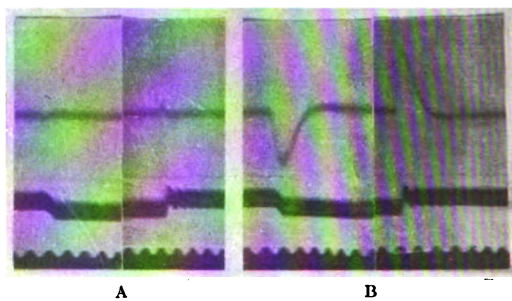


Fig. 12

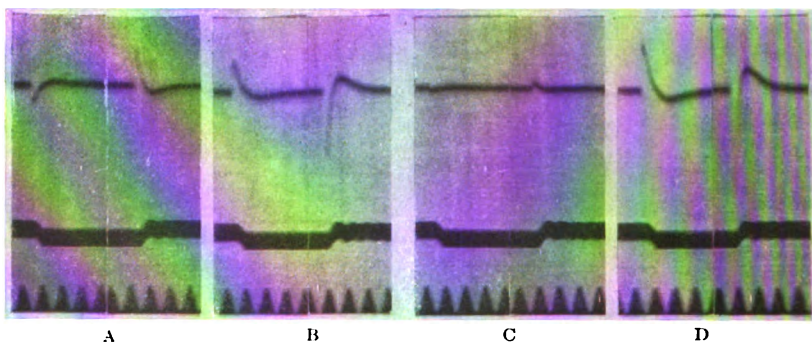


Fig. 11

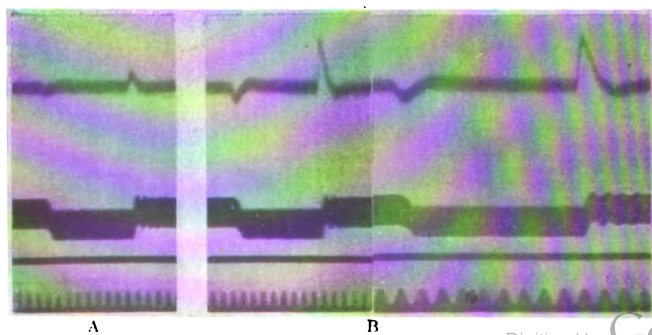


Fig. 13

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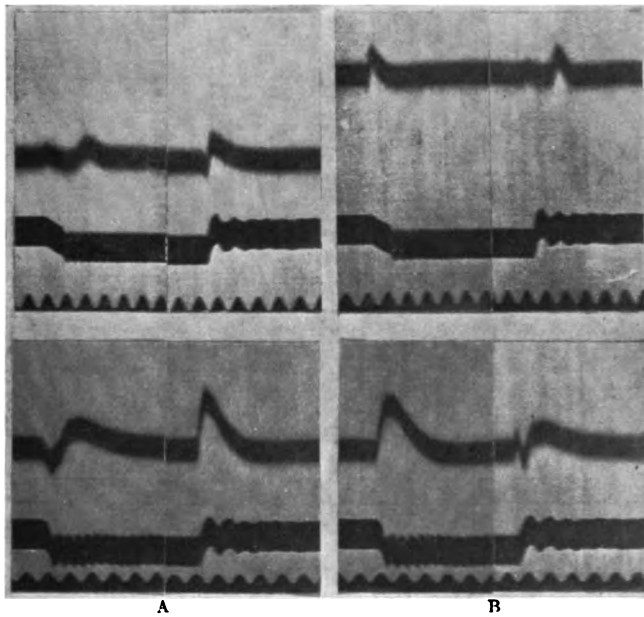


Fig. 15

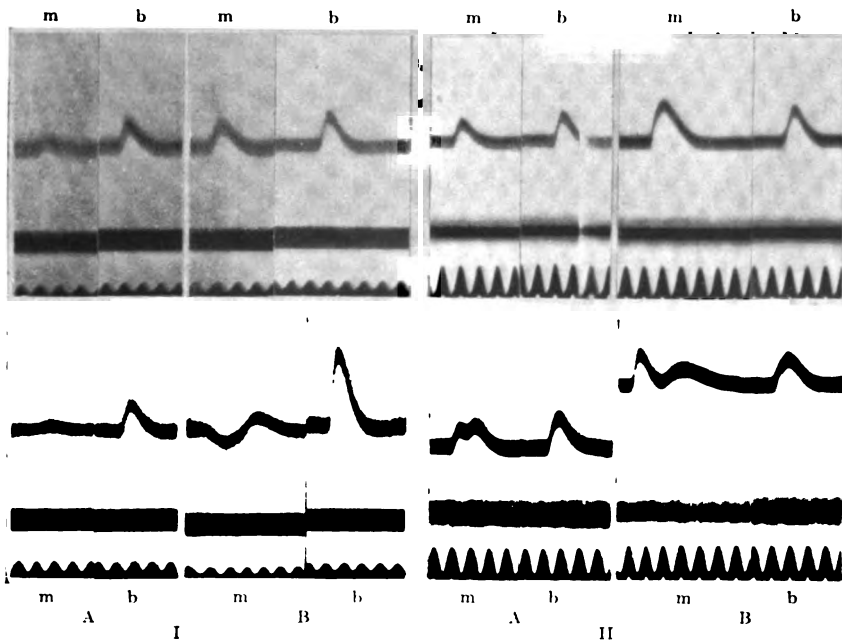


Fig. 16

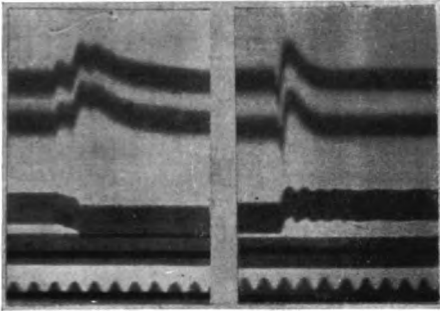


Fig. 14

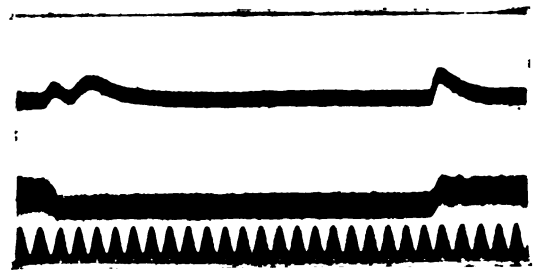


Fig. 17

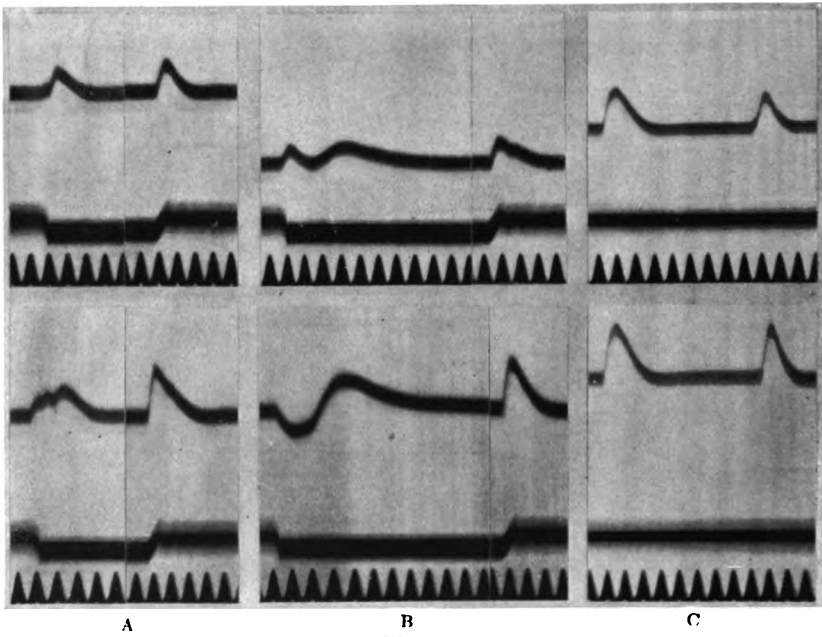


Fig. 18

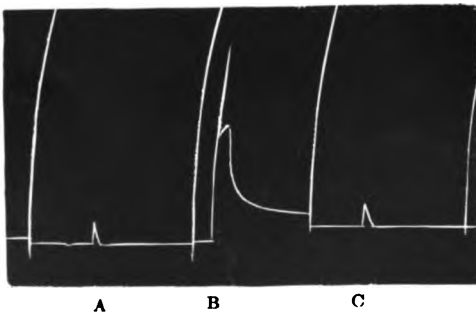


Fig. 19

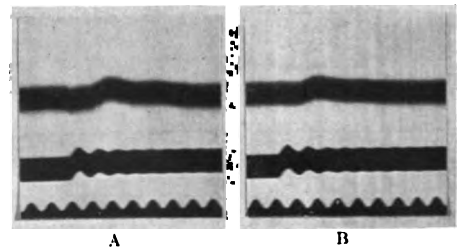


Fig. 20

ELECTRICAL STUDIES IN MAMMALIAN REFLEXES

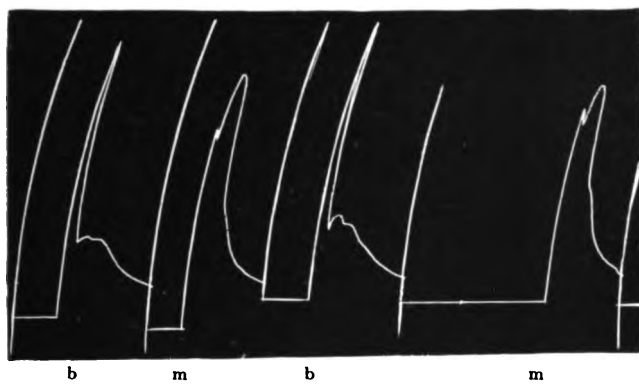


Fig. 21

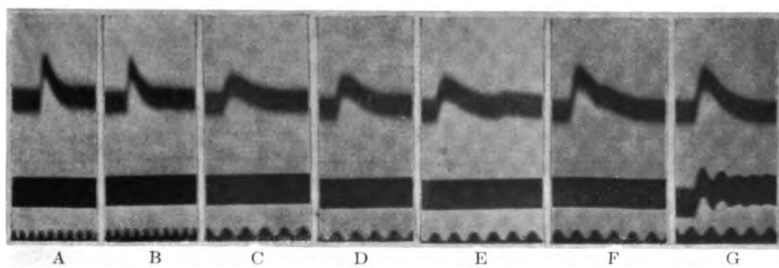


Fig. 22

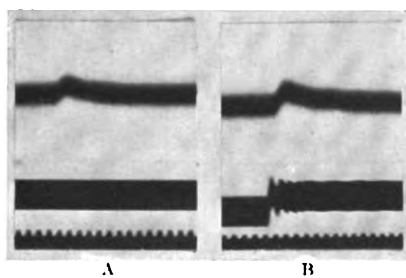


Fig. 23

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